THE NEUROHYPOPHYSIS

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Edited by
H HELLER

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COLSTON SYMPOSIUM ON THE NEUROHYPOPHYSIS 1956

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Foreword

THE name of Edward Colston, the great seventeenth century philanthropist and educationalist is associated in Bristol with a number of scholastic and charitable institutions It was adopted by a group of public spirited citizens when, in 1899 they established the University College Colston Society with the aim of fostering the young and struggling University College For a decade it played a part in the movement which culminated in the institution of the University of Bristol in 1909

The Society then changed its name and made its object more precise it became the Colston Research Society and devoted itself to the encouragement of original work in the University It made grants for the purchase of apparatus and for the other expenses of research As resources increased activities expanded and notably in the later thirties the Society financed a full scale Social Survey of Bristol

After the war a new reconsideration of policy led to the decision to devote the major part of the Society s efforts to the promotion of an annual symposium the first being held in 1948 The rapid growth of the symposium as a means for the advancement of knowledge is one of the remarkable features of the intellectual life of recent years Usually such meetings are fostered by bodies interested in one particular field of learning As the list of titles (on the page opposite) shows, no such limitation applies to the symposia of the Colston Research Society That the subject should be one at an interesting and active stage of development is the main factor in making a choice The fact that the symposium is held in one of the younger seats of learning with its home in an historic city, is a stimulus not only to the University but also we believe, to the visiting guests who have come from many countries The publication of the proceedings ensures the communication of the papers and discussions to wider circles

As President of the Society for the year 1955 56 it was my privilege to preside over the eighth symposium on The Neurohypophysis, the proceedings of which are printed in this the eighth volume of the Colston Papers

M SKENE

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Preface

This Symposium on the Neurohypophysis was the first international meeting devoted solely to the subject It mirrors not only the diversity of aspects of the theme but also the rapid progress in recent years in the study of this involved endocrine system This progress has been essentially twofold Firstly there is now strong suggestive evidence that the neurohypophysial hormones are elaborated not in the posterior pituitary lobe but in the hypothalamus and several of the papers read were concerned with the implications of this concept Secondly the chemical nature of the active principles—in some mammalian species at least—has been established However as Sir Henry Dale reminded us in his opening address the physiological significance of the neurohypophysial hormones is known only incompletely Admittedly the regulatory role of the antidiuretic principle in the water metabolism of mammals (and most likely in that of lower vertebrates) can no longer be doubted but the importance of the other pharmacologically demonstrable effects of posterior pituitary extracts is as yet based less securely While for example there is strong evidence that oxytocin is normally implicated in the complex mechanism of lactation its role in parturition is far from clear Moreover as is apparent from experimental results reported in this symposium the neurohypophysis may have some further physiological functions with which we are even less perfectly acquainted vasopressin may in certain circumstances be concerned with the regulation of blood pressure oxytocin may play a role in renal electrolyte excretion and may also be involved in the uterine mechanism for the ascent of spermatozoa

There is another unsolved (or only partially solved) problem which was frequently mentioned namely that of the estimation of the neurohypophysial hormones in body fluids This has been achieved reliably when the concentration of exogenous or endogenous hormone is relatively high but the normal blood levels of the posterior pituitary hormones in animals or man have not been established Until this has been done clinical research on neurohypophysial function will obviously be severely handicapped For this reason and not only because of the limited time papers on clinical aspects were deliberately excluded from the symposium But as this volume shows the subject was otherwise treated on a broad basis which included the comparative physiological approach

There remains the pleasant duty to thank the individuals and organizations that made this symposium possible First and foremost the Colston Research Society and in particular its President Professor M Skene and its Secretary Mr R H Brown whose help has been invaluable Secondly Sir Henry Dale and the Wellcome Trust for their generous grant which enabled us to invite speakers from the United States and Canada I am also much indebted to Dr S J Folley Chairman of the Society for Endocrinology and Dr G E W Wolstenholme Director of the Ciba Foundation for giving me their advice in the early stages of organizing the symposium and

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to Sir Lionel Whitby, Regius Professor of Physic and Master of Downing College, Cambridge, who accepted the onerous task of acting as Guest Speaker at the Reception of the Colston Research Society Finally, I should like to express my sincere thanks to Dr R J Fitzpatrick who acted as secretary to the symposium and who carried the main administrative burden to Miss A T Walker and her staff who looked after the members of the symposium at Manor Hall, to Dr M Ginsburg who acted as Recorder, to Miss G H Pope whose secretarial services have helped so much in the preparation of the manuscripts to Messrs J G Lane and K Lederis for technical help during and after the symposium and last but not least to the printers Messrs J W Arrowsmith Ltd

H HELLER

Bristol, 1956

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Evidence concerning the endocrine function of the neurohypophysis and its nervous control

by

SIR HENRY H DALE

THE 1891 edition of Michael Foster s then justly famous *Text Book of Physiology* which provided the basis for my own undergraduate studies of the subject when these began in 1894 was curtly explicit in its account of the knowledge then available concerning the functions of the pituitary body With regard to the purposes of the organ as a whole , it stated, we know absolutely nothing —a statement which you may imagine was not without an aspect of reassurance for a student with an examination in prospect And, when the first evidence to suggest a function for this previously mysterious organ, appeared a year later in 1895 this was concerned, as was still later to be made clear with only one of the parts of its composite structure namely with its posterior lobe the neurohypophysis which is the subject to which our discussions in this symposium are to be limited—except, of course in so far as some contributor may need to mention data which are more directly concerned with the anterior lobe—the adenohypophysis—or with the so called pars intermedia on account of light which they may throw indirectly upon the functions of the neurohypophysis and the mechanisms of their control which are on this occasion to be our sole concern

The first evidence then suggesting any function for the pituitary body and for the neurohypophysis in particular was presented in a short paper by George Oliver and E A Schafer (1895) This was in effect a supplement to their main paper on the remarkable effect on the circulation of an extract of the suprarenal gland—a potent action which they had seen and reported briefly in the previous year 1894 when Dr Oliver the enterprising physician from Harrogate had first persuaded Professor Schafer inclined to be impatient and incredulous to try the effect of injecting such an extract into a vein of an anaesthetized dog at the end of an experiment which Schafer had been making with a different object And having in their full paper in the following year given an extended account of this astonishing activity of a supra renal extract and having shown that the substance responsible for it was obtain able only from the medulla Oliver and Schafer were naturally curious to discover whether other ductless glands contained any comparable immediately active sub stances They reported the results of this survey in the paper which directly followed that on the suprarenal extract in the same number of volume 18 of the *Journal of Physiology* published in 1895 And there you can find this first account of the activity with intravenous injection of an extract prepared from the whole pituitary body this being the only one of the other glandular extracts which they tested which showed a pressor or indeed any other specific action on the circulation, comparable in intensity with that which they had observed with the suprarenal extract the effect

of the pituitary extract, though it was much the more persistent being the less impressive of the two because it was wholly due to an intense vasoconstriction, and showed none of the cardio acceleration which contributed so much to the suprarenal effect

It was W H Howell (1898) who, some three years later, showed that the substance responsible for this pressor action of a pituitary extract was obtainable only from one part of the organ, and, rather surprisingly, only from its posterior, neural lobe Howell also showed that when the prolonged pressor effect of a substantial first injection had subsided, the animal was almost completely insensitive to the action of a further similar or even larger injection, and remained thus refractory for a period up to several hours according to the size of the dose responsible for this tachyphylaxis In the following year, Schafer & Vincent (1899) confirmed both these observations They were puzzled as were others later by the finding, which they were nevertheless obliged to confirm, that such a powerful, immediate, pharmacodynamic activity should be due to something present only in the posterior lobe of the pituitary body It would have been much easier of course to credit the anterior lobe so obviously glandular in structure with the production of something so highly and specifically active whereas the posterior neural lobe, from which alone the activity could be extracted appeared to consist almost entirely of nerve fibres and neuroglia and to be in fact a kind of extension through the infundibular stalk from the grey matter of the floor of the third ventricle, though almost devoid of nerve cells Schafer & Vincent ascertained by experiment that otherwise similar extracts, made from grey matter from other parts of the brain did not in fact exhibit any activity of this kind And this apparent anomaly of the production from what looked like the almost purely nervous tissue of the posterior lobe, of an intensely active substance which no other nervous tissue yielded with the suggestion that nevertheless this part of the pituitary body like the suprarenal medulla ought to have an important endocrine function continued for a number of years to puzzle and to disconcert the minds of physiologists who became interested in the problem It seemed to be difficult for them to reconcile such a finding with their sense of physiological propriety so that they became too eagerly alert perhaps for any evidence which might appear to offer a plausible way of escape from so embarrassing a paradox A key to its meaning seems now at length to be offered by the novel and highly *suggestive lines of evidence which have been followed in recent years* concerning the relation of the structures of the posterior lobe to the nerve cells of the hypothalamic nuclei and the dependence upon this connexion of the hormonal content of the lobe And this appears likely to be one of the most important subjects for our consideration at this symposium Since my own part in our discussion can be little more than reminiscence and gossip concerning a phase in the evolution of its subject now long past I can contribute little to the consideration of this recent development except to emphasize the genuine difficulty which earlier investigators found in this idea that a tissue while it appeared to consist almost entirely of nerve fibres and neuroglia could also function as an endocrine gland—a difficulty which was certainly not diminished when it was found that the posterior lobe extract had other immediate physiological actions comparable in interest and intensity to the pressor activity which had first been noticed

Magnus & Schafer (1901) observed that an intravenous injection of the pituitary posterior lobe extract into an anaesthetized animal produced also, after a latency a conspicuous increase in the rate of the secretion of urine and, a few years later, this observation was confirmed and extended by Schafer & Herring (1906) It is of course, now generally recognized that this effect definite and striking though it is, and regularly as it can be reproduced under the given conditions is, in fact an anomalous and artificial result due to the depressed kidney function produced by the anaesthesia and to the use of the intravenous route for administering the pituitary extract in relatively large doses It was later recognized that the truly physiological *anti*diuretic effect now accepted as the genuine hormonal action on the kidney could only be reproduced artificially if the extract was injected hypodermically or in very small doses intravenously and into unanaesthetized animals

Before we discuss further this natural antidiuretic effect however, I ought to make brief mention of another action of the posterior lobe extract on the plain muscle of the wall of the uterus which I first described incidentally (Dale, 1906), having come across it almost by accident when I recorded the already known pressor action for another purpose I was studying a now familiar but then newly discovered action of certain alkaloids of ergot—what has since been termed a sympatholytic action—in which augmentor actions of adrenaline and corresponding effects of sympathetic nerves transmitted as we now know by the release at their endings of adrenaline and its primary homologue are suppressed or replaced by inhibitor effects In a female cat at an early stage of pregnancy I happened to be recording the arterial blood pressure and the activity of the muscular wall of the uterus and by a previous dose of an ergot preparation, the actions of adrenaline on both of these had been so reversed that each injection of it now produced a fall of the arterial pressure in place of the normal rise and a relaxation of the uterus in place of the previous contraction I thought that it would be of interest to know whether the pressor effect of the pituitary posterior lobe extract would also be reversed like that of adrenaline or as I expected unchanged When accordingly I injected an appropriate dose of the pituitary extract I observed that it had not only retained its normal pressor action, in contrast to the reversed depressor action of adrenaline but that it also produced a powerful contractile response of the uterus not hitherto described Other experiments made then to study this action deliberately (Dale 1909), showed that it was produced on the uterus in all species and in various physiological conditions even in those in which the natural effects of adrenaline were to produce simple inhibition of the organ s tone and rhythm It was clear then, that these peripheral actions of the pituitary posterior lobe extract unlike those of adrenaline had no relation to the effects of impulses in the sympathetic or any other autonomic nerves And I took the opportunity to see also for myself the diuretic action on the anaesthetized animal of which a full description had then recently been given by Schafer & Herring (1906) also to make some observations on the nature of the substance assumed then to be only one which was responsible for all these actions, showing it to be excreted in the urine, stable to peptic digestion and to boiling at a mildly acid reaction but readily destroyed by tryptic digestion or by hot alkalis and to have, accordingly the characters of a relatively simple polypeptide For the time being it seemed to me natural to suppose in default of good

evidence to the contrary that one and the same substance was responsible for all these different actions, in accordance with the well known logical maxim attributed to William of Occam—*entia non sint multiplicanda praeter necessitatem*

We may leave that point for the present, noting only that in its further discussion we should also include the substance in the extract responsible for the milk ejecting galactogogic effect on the mammary gland, later recognized by Ott & Scott (1910) Meanwhile we must give some further consideration to the really physiological action of the extract on the function of the kidneys This first came to light through a sequence of clinical observations on human patients I think that we may suppose that the demonstration by Schafer and his co workers of the anomalous diuretic effect on anaesthetized animals, to which I have referred, had some influence in directing the attention of J E Frank (1912), to a possible connexion between diabetes insipidus and disease, or injury, of the hypothalamus, of the infundibulum, or of the neurohypophysis Frank having discovered strong pathological evidence of such an association appeared to find it natural to attribute the effect to an excessive outpouring of the supposed diuretic hormone evoked by irritation of the neuro hypophysis His observation however, seems to have stimulated two independent investigators, von den Velden (1913) and Farmi (1913) to try the therapeutic effect of hypodermic injections of the posterior lobe pituitary extract on cases of diabetes insipidus, and both of these observers reported a result which appeared to be the direct opposite of what might have been expected if Schafer s conception of diuresis as the normal effect of the extract and Frank s application of this idea to his patho logical findings had represented the true physiological function of the neuro hypophysis And it may perhaps, have some slight interest to recall, in passing that the successful treatment of diabetes insipidus by such injections, repeated at suitable intervals, had become an item of accepted, almost of conventional, therapeutic routine some eight or nine years before the discovery of insulin made possible a much more famous therapeutic advance in the successful treatment of the much commoner and much more tragically serious condition diabetes mellitus The preparation of an active extract from the pituitary posterior lobe had of course no such long history, of frustration and ultimate success as had been involved in that of the extraction and stabilization of insulin No more complicated method had been needed to obtain the antidiuretic hormone than the simple boiling of the posterior lobe substance with weakly acidulated water, and preparations so made had in fact been already available for several years for use in obstetrics so that they were ready to hand when their remarkable value in diabetes insipidus was discovered

Meanwhile the mode of origin and the precise physiological source of the active substance, or substances seemed to be still in doubt The results of operative re moval of the neural lobe by a number of different observers over a period of years using different operative methods and different species seemed to be difficult to interpret The finding for example that removal of the neural lobe alone or section of the infundibular stalk, caused in certain species only an evanescent diabetes and that when this had thus subsided extracts from what had been regarded as hypo thalamic brain substance were hormonally active, seemed for a time to make the issue only more complicated I must not attempt to anticipate what those with direct experience of this aspect of the problem may be going to contribute to the

symposium Perhaps I may venture however, to suggest that it seems now to be recognized that we ought not to restrict our conception of the hormone producing neurohypophysis to the rounded lobe which hangs as it were, at the end of the stalk, apparently we should include also, in the neurohypophysis tissue in the stalk and in the median eminence, which had earlier been assumed to be nervous and therefore not or at least not normally endocrine in function although as we have seen the almost entirely nervous nature of the tissue of the lobe itself together with its normal yield of a highly active extract, had long made it difficult to regard nervous structure and endocrine function as entirely incompatible

There appeared meanwhile to be a paucity of more direct evidence in favour of a supposition which is obviously of central importance to the whole direction and form of our discussion, namely that the highly active substance (or substances) which the neurohypophysis yields to artificial extraction are also naturally secreted from it as hormones There are others present with far better and more direct qualifications than mine for dealing with the evidence by which this important conclusion can now be justified Perhaps however I may allow myself to make special mention of a line of evidence which has developed during a period extending now over rather more than thirty years and which began with experiments in which the late E H Starling and E B Verney employed an acute form of physiological technique Starling & Verney (1925) perfusing the isolated kidney of a dog with blood from a simple heart lung preparation showed that it soon began to secrete a voluminous and highly dilute urine entirely similar to that of diabetes insipidus but that the addition to the artificially circulated blood of a small dose of the pituitary posterior lobe extract was sufficient to restore to the kidney its normal reconcentrating function And then in the following year after Starling s death Verney (1926) included also, in the heart lung circulation the isolated head of the dog and showed that this sufficed to enable the perfused kidney to continue the production of a urine which was normal in volume and concentration until the pituitary body was removed from the perfused head when the voluminous and dilute urine of diabetes insipidus soon appeared To me this seemed to be the most direct and convincing evidence till then available not only of the perfused kidney s requirement for the production of a normally concentrated urine of a substance present in an artificial extract of the neurohypophysis but also and most convincingly of the active secretion of that substance from the neurohypophysis into blood which was circulated through it In other words, it seemed to be at least as complete a proof of a normal endocrine function for the neurohypophysis as any which had been given or indeed has since been given for any other organ And you will I am sure be familiar with the series of elegant experiments on normal conscious animals which have since enabled Verney and his co workers including Dr Pickford whom we shall hear later to study in detail the conditions by which this endocrine activity of the neurohypophysis is naturally excited and controlled and of the part played, in that excitation and control by emotional factors, acting upon the neurohypophysis through the nuclei of the hypothalamus

I must also say something more directly about the question already mentioned by implication whether these different effects of the neurohypophysis or of artificial extracts from it, are due to only one or to more than one hormone, or active principle

evidence to the contrary, that one and the same substance was responsible for all these different actions, in accordance with the well known logical maxim attributed to William of Occam—*entia non sint multiplicanda praeter necessitatem*

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able to tell us whether any comparison has yet been made of the ratios between pressor and antidiuretic activity in these two different vasopressins, containing arginine and lysine respectively and, if so, with what result

With the investigation of the related but essentially different question whether these different active polypeptides exist separately in the living gland or as components of a single multivalent macromolecule, I have had no direct concern Professor van Dyke as we all know obtained in a press juice from the fresh substance evidence of a protein moving as a unit under electrophoresis separable by differential centrifugation and yielding both types of activity to extraction apparently in the natural proportions I should have been more impressed by this evidence if I had not myself had bitter experience over a wide interval of years of the tenacity with which the two active principles from the boiled extract in which we now know that they exist separately adhere even to well crystallized precipitates and apparently in proportions not obviously different from those in which they were present in the original extract Two distinguished chemical colleagues of mine the late George Barger and the late Harold Dudley suffered separate and harrowing disappointments at an interval of many years Each of them in turn obtained from the extract after months of careful work a well crystallized salt showing the different activities with a high intensity and in apparently unchanged proportion and each in turn not unnaturally thought for a time that he had prepared a pure salt of a single multivalent principle A more careful scrutiny of details however especially of the *degree* of such activity per unit of weight induced each of them in turn to apply further methods of purification with the result that the crystalline salt proved in the one case to be a picrolonate of lysine and in the other a double picrate of potassium and creatinine To each of these salts both the active principles had adhered with an astonishing tenacity evidently only in traces but these had been sufficient to endow the crystals with such misleading activity I am accordingly a little disposed to doubt whether we can safely assume that even what seems to be an electrophoretically uniform protein from the fresh gland substance might not retain sufficient of these obstinately clinging polypeptides by mere physical adhesion to create the impression of a single multivalent macromolecular hormone I am very much hoping that Professor Fromageot is going to throw some further light on this question for us The really important question for the physiologist however is whether these active substances can be separately released from the living lobe in response to the appropriate stimuli or must always be shed into the circulation together as components of the suggested macromolecule Teleology of course would strongly favour the probability of separate release in response to specific needs but teleology is a dangerous guide and as Starling once said to me one must always suspect Nature of setting booby traps

And then we shall certainly have discussion—and I look forward to the opportunity of learning much from it—concerning the relation between the neurohypophysis with its specific hormone or hormones and the nuclei in particular the supraoptic and paraventricular nuclei of the hypothalamus This aspect of our subject seems to me to have a very special interest on account of the suggestion which seems to emerge from the evidence of a relation which so far as I know is the first of its kind to be recognized in any endocrine system of the higher vertebrates I know that

I am not going to revive the details of a controversy, in which my late colleague Harold Dudley and I found ourselves most unwillingly involved with a very greatly revered old friend of mine, the late Professor Abel I only wish to emphasize the fact that our own experiments were concerned throughout with one limited question, namely, whether the different activities of the ordinary, boiled extract of the posterior lobe substance were due to one common active principle, as several of us had earlier supposed, or to at least two different principles, responsible for different features of the complex activity Dudley (*1919*) *made what seemed to me an unquestionable*, though incomplete separation of the oxytocic from the vasopressor constituent using a method of continuous extraction from the weakly acid watery extract with a partially miscible, neutral solvent, normal butyl alcohol The method, indeed was similar in principle to that with which du Vigneaud *and his team, during years still* quite recent have eventually achieved so complete and astonishing a success It will be remembered also that, in the intervening period, Kamm and Aldrich and their co workers (1928), in the laboratories of Parke Davis and Company had worked out a method for separating these two principles on a manufacturing scale, so that they could be offered for general use in separate solutions, with activities sufficiently pure for practical, therapeutic purposes And now of course the brilliant success of du Vigneaud and his team has settled this question of one or two active principles in the extract, beyond all further discussion Using the modern resources of counter current distribution and chromatography, they have succeeded beyond all expectation, not only in isolating both the oxytocic and the vasopressor principle, each in a state of sufficient purity to enable them to determine completely its complex cyclo polypeptide constitution but even in confirming that of the oxytocic principle by artificial synthesis It is a disappointment to us all that Professor du Vigneaud has not been able to come over and join us in this symposium We should all have welcomed the opportunity of expressing to him directly our very great admiration for a truly astonishing achievement The work of Professor van Dyke and others who have given their co operation in physiological experiments, allows us I think, further to accept, as now also beyond the need for discussion the fact that the main oxytocic action and the galactogogic action of the extract are both due to one of the two substances and that the pressor and antidiuretic actions are both due to the other which *also has intrinsically not from imperfect separation a minor oxytocic activity of its* own There are probably other points of interest about these two active substances about which we may hope to hear in our discussions Some years ago Professor Heller (1939) produced evidence, which seemed to show that the pressor activity of an extract from the neurohypophysis was less stable to heating at any reaction than the antidiuretic activity, and that a preparation could even be obtained which had no pressor but retained some antidiuretic activity A difference of sensitiveness between the two types of reaction may be involved but I have wondered if possibly the amino acid structure of the natural pressor antidiuretic principle might undergo a minor change involving an unequal loss of the two types of activity and whether the constitution of this hormone might even undergo some change in the gland during growth from infancy to maturity Du Vigneaud and his team have already discovered an important difference between the constitution of the vasopressin from the bovine and of that *from the porcine posterior* lobe, and perhaps somebody may be

and possibly, other classes of invertebrates And I think that I need not further emphasize the relief which these recent developments offer, from the embarrassments experienced by earlier workers and from the unprofitable diversions of experiment and speculation which were caused by their efforts to evade what they regarded as an incompatibility—an assumed incompatibility between an essentially nervous structure and a content of substances with activities so intense and so specific, that it was difficult not to credit them with important functions as hormones

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the organizers of the symposium had been hoping that we should have with us some of the American colleagues, such as Professor Scharrer and Dr Bodian, who have made such important contributions to this chapter of our subject, but who to our regret have not been able to come Fortunately we have with us, in Professor Bargmann of Kiel and Professor Hanstrom of Lund, investigators who have been among the pioneers in the exploration, in different directions of this rapidly opening field of knowledge So far as I am aware there is no other instance yet known in the endocrine systems of higher vertebrates, in which not only the output of a hormone from an endocrine gland but also its production and maintenance there are under such complete and immediate nervous control, as seems undoubtedly to exist in this case The severance of the nervous connexion, between the neural lobe and the hypothalamic nuclei leads to the disappearance of the hormones from the lobe within a few days There have, further been most suggestive descriptions of a specifically staining material, sheathing the endings in the lobe of the axons from the cells of the hypothalamic nuclei which form indeed so large a constituent of its fabric, and we shall hear I think that material with the same staining properties has been traced along the whole course of these axonal fibres, right back to the nerve cells of the hypothalamic nuclei in which they originate I hope that it is not too fantastic to find in the relation thus suggested, an analogy not too remote to the case of the ordinary efferent nerve fibres as found in the peripheral nervous system I think that we can now take it to be accepted that the effects of impulses in these fibres are transmitted to the effector cells in close contiguity with which they have their endings by the release from those endings of one or another specific transmitter substance accumulated there indeed in readiness for such release, but traceable also in minor amounts along the whole course of such fibres, back to the nerve cells in which they originate And, here again the transmitter disappears in a few days and completely, from any part of such a fibre including the depot at its ending when it is separated by section from its nerve cell of origin And, if we can accept the suggested method of the formation maintenance and release of the neurohypophysial hormones as also dependent on a maintained continuity with the cells of the hypo thalamic nuclei we seem to have at least a suggestive analogy with these peripheral nerve fibres and their specific transmitters with the very important difference, of course, that the specifically stimulating substances released from the endings of the nerve fibres in the neurohypophysis do not act as immediate transmitters of the effects of nerve impulses to contiguous effector cells or secondary neurones but as hormones carried in the blood stream to the plain muscle and epithelial cells of distant organs on which they then produce at this long range their specific stimulating effects

I have already stated that so far as I am aware this is the only case which has yet been discovered in the higher vertebrates and especially in the Mammalia, from which so large a part of the evidence has been obtained, in which substances with a truly hormonal function have been found to be thus produced by essentially nervous structures Apart however from certain mechanisms for controlling the activities of chromatophores in some cold blooded vertebrates I expect that Professor Hanstrom will be giving us an account of some at least of the now numerous examples available of suggestively analogous neuro hormonal mechanisms in insects crustaceans

Relationship between neurohypophysial structure and function

by

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AT the turn of the century, the application of a variety of histological techniques revealed the most important structural elements in the posterior lobe of the hypophysis Thus in 1894, Cajal (1911) noted that fine nerve fibres were remarkably abundant in the posterior pituitary he claimed that these fibres had their origin in a group of cells situated behind the optic chiasma

He reported the presence of other cells which he did not identify embedded in the plexus formed by these nerve fibres cells which later workers have interpreted as glial cells The capillaries of the neurohypophysis with their associated argyrophil connective tissue form a third and essential component of the gland These were not described until some twenty years after Cajal s original paper

At that time it was impossible to make any reasonable functional interpretation of the so called pars nervosa Neither clinical nor experimental observations pointed unequivocally towards any specific role for the neurohypophysis that is towards any role which could account for the abundance of nerve fibres and glial tissue it contained This is not to say of course that there were not claims that substances extracted from the posterior pituitary of certain animals had specific humoral properties capable of causing for example an elevation of the blood pressure At this stage Biedl (1913) made an important point namely that it is extremely difficult to make a complete separation of the pars nervosa from the pars intermedia in such a way as to be quite certain that the posterior pituitary tissue is completely free of epithelial cells Workers in this new field of endocrine research were inclined to focus their attention on epithelial rather than on nervous tissues since at that time at the beginning of the century all the organs of known endocrine activity were epithelial in structure and we can thus readily understand why Biedl (1913) in his standard work on the endocrine system left open the question of the function of the posterior lobe although he was ready to include the anterior lobe and pars intermedia of the pituitary among the endocrine organs

However the morphological investigation of the neurohypophysis received a new stimulus when it was shown that Biedl s classification was untenable and that the posterior pituitary had indeed a specific endocrine function

This was first established by Geiling and his co workers (1937) who studied animals in which the pituitary lacks a pars intermedia and in which a connective tissue septum separates the adenohypophysis from the neurohypophysis From the posterior

We must bear in mind at this point that the recognition of hormone in a tissue culture is no proof in itself of the formation of hormone by that tissue it could merely indicate the persistence in that tissue of a store of hormone

Now Geiling & Lewis (1935) made their investigation on cultures at their most six days old Hild (1954) has been able to show that hormone contained in tissue cultures from the mammalian posterior pituitary disappears within ten days and that following this no hormone is elaborated by pituicytes in spite of the fact that they remain viable Further, no active substance can be extracted from explanted posterior pituitary if taken from an animal in which the posterior pituitary has been depleted of hormone by physiological means Hild claims also that the morphological behaviour of pituicytes *in vitro* suggests that these cells are not secretory cells but rather are protoplasmic astrocytes modified by their situation

In short we must regard the nerves of the posterior pituitary as the source of the posterior pituitary hormones, as Gaupp (1941) among many others has insisted on These nerves form a thick network composed of fine unmyelinated fibres which in silver preparations are remarkable for their numerous nodular thickenings In the vicinity of blood vessels these fibres form a particularly fine meshed plexus Their endings clearly do not form synapses with pituicytes as they should do, if the innervation of pituicytes were necessary for their secretory activity To understand the function of these fibres we must consider their origin We have to take into account axons the cell bodies of which are, in part ganglion cells in the nuclei of the tuber cinereum but the majority of nerve fibres in the posterior pituitary are derived from the cells of the supraoptic and paraventricular nuclei By and large therefore we can look upon these fibres as the terminations of the supraoptico hypophysial tract

These cells have in their cytoplasm granules or droplets of a colloidal substance which can be regarded as neurosecretory material The secretory function of the nerve cells was first recognized by E Scharrer and his co workers (1954*a* *b*) but only in the proximal part of the neurosecretory pathway Now however it is possible to demonstrate this neurosecretory material in the entire distribution of the supra optico hypophysial tract, from the nuclear region (Plates I II and III) to where the fibres fan out in the posterior pituitary (Plate IV) This has been achieved by a variety of staining and histochemical technique which selectively demonstrate neurosecretory substance within the neuroplasm, namely the chrome alum haematoxylin technique (Bargmann 1949) the paraldehyde fuchsin technique (Gabe 1953 Dawson 1952) the thioglycollate ferric ferricyanide reaction for cystine (Sloper 1955) and the performic acid Alcian blue reaction for cystine (Sloper & Adams 1955) (Plate V) With these methods one can see that the colloid droplets called Herring bodies which were once thought to lie free in the posterior pituitary are in fact swellings on neurosecretory fibres (Plate VI) Apart from them we find everywhere fibres characterized by smaller thickenings, that is beaded fibres resembling strings of pearls We also find granules and droplets of secretion which are free in the interstitial space of the neurohypophysis and which are not apparently related to nerve fibres

As for the manner in which neurosecretory products are released into the blood stream it is not yet clear whether they normally pass into the capillaries of the posterior pituitary (Plate VII) in the manner suggested by Hanstrom (1952) and others

pituitaries of these animals these workers were able to extract oxytocin and vasopressin It cannot for a moment be supposed that these extracts were derived from adenohypophysial tissue Oldham s (1938) observations are equally unequivocal She studied *Dasypus*, an animal in which the posterior pituitary is sharply divided from the adenohypophysis, and she found that the posterior pituitary contained antidiuretic as well as pressor and oxytocic activity

These observations indicated that the neurohypophysis had a specific hormonal function The question arose as to which structural elements were responsible for the production of the hormones In answer to this question we can say that neither the glial cells of the posterior pituitary nor its nerve fibres, nor both together, represent the site of formation of the three posterior pituitary activities

The problem has been investigated in a number of ways in particular with the aid of tissue culture There have at the same time been further morphological studies which have amplified the original findings of Cajal

Now, first, with regard to the glial cells of the neurohypophysis Bucy (1932), who named these cells pituicytes, put forward the theory that they were responsible for the formation of the posterior pituitary hormones Their secretory activity was corroborated according to Griffiths (1940), by the observations of Geiling & Lewis (1935) who found vasopressin in tissue cultures of the posterior pituitary of the mouse These observations were confirmed by Andersson & Haymaker (1935) Griffiths held that in such explants of posterior pituitary tissue the nerve fibres had degenerated and therefore, by exclusion, only the pituicytes could be regarded as the source of the posterior pituitary hormones Romeis (1940) endorsed this view He stressed the presence of foamy vacuolation of karyorrhexis and of karyolysis, which histological changes he regarded as evidence of holocrine secretion Independently Gersh (1939) had also described what he termed glandular cells in the neurohypophysis of the rat, which were filled with granules and which he held to be the site of formation of antidiuretic hormone Indeed I myself believed in the secretory nature of these glial cells for their nuclei contain large spherical inclusions which I interpreted as evidence of a secretory function

The nerve fibres which run between the diencephalon and the neural lobe were held by Gersh (1940) to regulate the release of the antidiuretic hormone In this respect Gersh s views are in accord with those put forward by Ranson (1938 1939) and his school These workers studied the development of experimental diabetes insipidus following the interruption of the supraoptico hypophysial tract They attributed this condition to the destruction of the secretomotor innervation of the pituicytes

However, as Hild (1954) has recently noted there is one difficulty inherent in any attempt to combine Ranson s hypothesis with the views put forward by Griffiths, Gersh and other workers According to Ranson, interference with the secretomotor innervation of the pituicytes leads to the cessation of hormone production Griffiths on the other hand held that the pituicytes in tissue culture had an intrinsic secretory activity, and stressed the fact that the supposed secretomotor fibres in such explants were no longer viable In view of these difficulties it seemed important to repeat the older tissue culture experiments so as to make a critical appraisal of this problem of hormone production *in vitro*



Plate I

Paraventricular nucleus, dog. Nerve-cells stained selectively with chrom-alum haematoxylin (Gomori) 90×

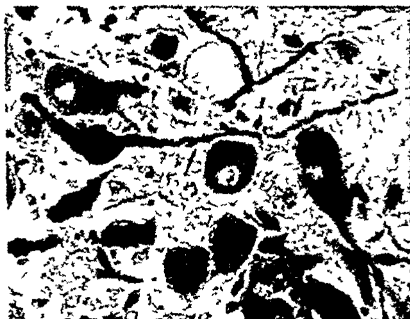


Plate II

Group of neurosecretory cells (nucleus supraopticus, dog). Note the nerve fibres. (Chrom alum-haematoxylin Gomori) 640×

In a preparation stained to demonstrate neurosecretory material it can be shown that the neural lobe contains much more of this substance than any other part of the neurosecretory pathway. This suggests that the posterior pituitary stores the material. A point much in favour of this view is that one can virtually empty the pituitary of neurosecretory substance in a wide variety of ways, for example by dehydration, (Ortmann, 1951), adrenalectomy (Fichner, 1953) or stress. With return to a normal allowance of drinking water after dehydration the posterior pituitary fills once more with neurosecretory substance. In Hild & Zetler's (1953) experiments this was shown to take some days.

Such observations raise a number of interesting problems. First, how does neurosecretory material accumulate in the neurohypophysis? Secondly, where does this material come from? and thirdly, what is its functional significance?

There is a good deal of evidence to suggest that neurosecretory substance is formed in the supraoptic and paraventricular nuclei and is transported down the neurosecretory pathway, *the supraoptico hypophysial tract, to its depot organ, the posterior* pituitary. The same appears to be the case in invertebrates, for example in the intercerebral corpus allatum system of insects and in the X organ sinus gland *system in the eyestalk of Crustacea (Berta Scharrer, 1951, 1952a, 1952b, 1952c, 1952d;* B. & E. Scharrer, 1944, 1954; Hanstrom, 1953). Further, in a large number of vertebrates from mammals down to Amphibia, operative interference with the neurosecretory pathway leads to the proximal accumulation of neurosecretory substance and to a diminution in the amount of this material in the posterior pituitary. A similar accumulation has been observed in the human pituitary stalk by Muller (1955) following compression of the infundibulum by a meningioma and by Sloper (1955) following hypophysectomy for the relief of malignant disease. Finally it is of interest that Hild (1954) has observed and filmed in tissue culture the transport of granules in a distal direction down the axones of living supraoptic cells in the dog. A point which presents some difficulty is that in the developing foetus stainable neurosecretory material is first demonstrable, not in the supraoptic and paraventricular nuclei, but in the neurohypophysis. This observation does not in my opinion weaken the theory of the transport of neurosecretory substance. For it seems to me that the granules of neurosecretion probably require an initial period of maturation, only after which they become visible in the light microscope.

It is difficult to be dogmatic about the chemical nature of neurosecretory material. Schiebler (1951, 1952) supposed it on histochemical grounds to be a glycolipo protein complex. Barnett & Seligman (1954) with their dihydroxyl dinaphthyl disulphide reaction observed that it was rich in cystine, a point made independently by Sloper (1955) with the thioglycollate ferricyanide reaction. For this and other reasons, these workers claim that the neurosecretory substance is essentially a protein. The supposedly high content of cystine in this substance is of some interest in view of the fact that posterior pituitary extracts are also very rich in cystine.

This brings me to the question of the functional significance of neurosecretory material. Whether we regard this material as representing the posterior pituitary hormones themselves, or as the carrier substance for these hormones, it is clearly necessary to prove that there is a close relationship between the two, that is, between what we can *stain* and what we can *extract*. This of course requires that oxytocin



Plate V

Nucleus praeopticus of the tench (*Tinca vulgaris*) stained with Alcian blue (Sloper's method) Nuclei of the nerve cells unstained 615 ×



Plate VI

Herring body with protuberances dog (Chrom alum haematoxylin Gomori) 1 400 ×

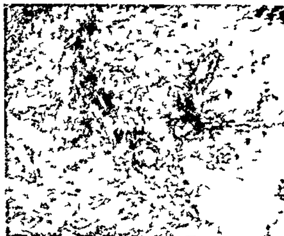


Plate VII

Neurosecretory material surrounding capillaries of the neural lobe (dog chrom alum haematoxylin) 150 ×

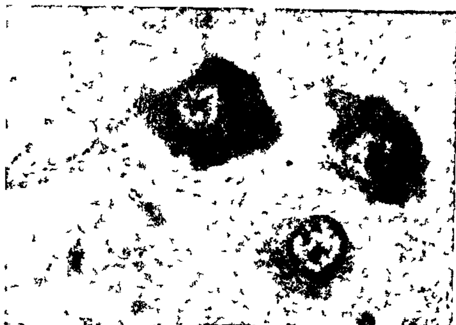


Plate III

Neurosecretory cells (nucleus supraopticus dog) showing different amounts of neurosecretory substance (Chrom alum haematoxylin Gomori) × 200 ×

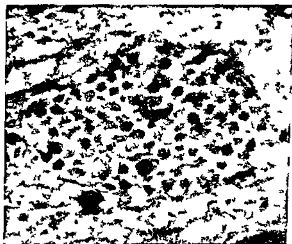


Plate IX

Electron microphotograph of a single nerve fibre in the neural lobe of the cat containing many granules (Fixation osmic acid) 23 000 ×

vasopressin and antidiuretic hormone can be found not only in the posterior pituitary, but also throughout the whole neurosecretory pathway from the nuclear region to the neural lobe

In this connexion we must recall the investigations made long ago by Trendelenburg (1928), and Sato (1928) and Melville & Hare (1945) concerning the presence of posterior pituitary hormones in the hypothalamus These investigations were based on material the exact source of which in the brain is rather obscure However Hild & Zetler (1951) made extracts from pieces of brain which contained little tissue save the ganglion cells of the supraoptic and paraventricular nuclei and fibres of the supraoptico hypophysial tract These extracts contained material with oxytocic antidiuretic and vasopressor activity as shown by the customary methods of biological assay On the other hand extracts made from neighbouring brain tissue which contained no neurosecretory material were inactive Further there was a direct relationship between the amount of neurosecretory substance and the biological activity of the tissue This was found to apply also to the accumulations of neuro secretory material which as I have already noted are to be found above the cut end of the pituitary stalk, for the hormone content in such regions is higher than would normally be in extracts made from these regions It is of interest too that compara tive studies have established a relationship between the amounts of neurosecretory material and the amounts of the hormones in the diencephalon of the dog pig and man (Hild & Zetler, 1952) Thus the dog is rich in neurosecretory substance and a large amount of hormone can be extracted compared with man in whom both the amounts of neurosecretory substance and hormones are low

Again, Heller s, (Heller 1947 Heller & Zaimis 1949) observation, that the posterior pituitary of the newborn contains only a small amount of antidiuretic hormone has its histological equivalent in the scantiness of the amount of neuro secretory material which can be demonstrated in this organ shortly after birth We must not forget, too the many and repeated experiments in which it has been shown that dehydration is accompanied by the disappearance of neurosecretory material from the posterior pituitary It is reasonable to correlate this with the observation that there is a fall in the amount of vasopressin which can be extracted from the posterior pituitary after dehydration (Ortmann 1951 Leveque & Scharrer 1953) Finally I must refer to the diminution in the amount of neurosecretory material which accompanies lactation This observation may be connected with the release of the milk let down factor that is perhaps with the secretion of oxytocin (Stutinsky 1953 Malandra 1955–56) Recently Vogt (1953) has raised the interesting question as to whether the hormone content of the material secreted in the hypothalamus alters in its composition during its transport to the neurohypophysis This is of course primarily a pharmacological rather than morphological problem Her own idea is that oxytocin which is only present in scanty amounts in the hypothalamus is either formed during the passage of this material down the supraoptico hypo physial tract or else that it is first formed in the posterior pituitary where it is added to the material formed in the hypothalamus

To sum up it seems reasonable to regard the neurosecretory neurons in the hypo thalamus as responsible for the formation of adiuretin oxytocin and vasopressin while the terminations of these neurons in the posterior pituitary may be regarded

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as the site of hormone release The distribution of these nerve endings in the pituitary of the opossum conforms extremely well with this conception in this animal, as Bodian (1951) noted the posterior pituitary shows a remarkable accumulation of neurosecretory material within the rod like nerve endings (palisade zone) at the periphery of the neurohypophysial lobules

Hormone can be rapidly released from the posterior lobe that is, in a matter of minutes This was well demonstrated by Verney s (1947, 1948) elegant experiments in the dog He showed that variations in the osmotic pressure of blood reaching the head were followed by the rapid release of adiuretin from the posterior pituitary

Verney s experiments imply the immediate stimulation of the neurosecretory cells in the hypothalamus by these variations in osmotic pressure, and in this respect the remarkably close contact between these cells and their capillaries may be significant, his *experiments also imply the transference of an impulse down the supraoptico* hypophysial tract to its endings in the posterior pituitary We do not know whether the pituicytes participate in the release of hormone from these endings From studies made with the electron microscope by Palay (1955) the capillaries of the posterior pituitary seem to be well adapted for the transfer of material through their walls for their endothelium contains submicroscopic pores, which until now have only been described in the capillaries of the renal glomerulus

Finally I feel that for the sake of completeness I must briefly mention the part played by the supraoptico hypophysial tract in the innervation of the pars intermedia and the pars tuberalis Neurosecretory fibres do undoubtedly enter the pars intermedia an organ indeed which is rich in glial tissue This applies particularly to fishes but also to some mammals As a result it is impossible to make a sharp distinction be tween the pars intermedia and the neurohypophysis Neurosecretory fibres also come into contact with the special vessels of the pituitary stalk, from which the blood passes into the pars tuberalis Occasionally such fibres can be seen to cross over into the pars tuberalis

These observations lead one to suppose that the hormones formed in the supra optic and paraventricular nuclei may have some influence on the pars intermedia and also perhaps on the pars distalis This seems to me to be an interesting and pro fitable subject for future research Such problems certainly make it only too clear that the structure and the function of the neurohypophysis are considerably more complicated than we used to suppose

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Bargmann I remember only Hild & Zetler s (*Pflugers Arch ges Physiol* **257**, 169 1953) experiments in the dog

Dale Do you know whether this stainable material represents one hormone alone or whether it is a substance with all the activities?

Bargmann I want to be very careful about this I think that the substance we can see is a carrier

van Dyke I think that one such investigation has been done by Simon (*Amer J Physiol* **107** 220 1934) or by Simon & Kardos (*Arch exp Path Pharmak* **176**, 238 1934) They found that after prolonged deprivation of water there was decrease in both hormones These observations were made in rats

Jewell Professor Bargmann did mention that during lactation it has been shown that there was a depletion of stainable neurosecretory material Has there been any differentiation between the paraventricular and supraoptic nucleus in this regard? Does it disappear from both?

Bargmann The results were very variable Sometimes it appeared that the supraoptic nucleus contained more of the material than the paraventricular and vice versa There are comments by Malandra (*Z Zellforsch* **43** 594 1956) on this point

Heller You mentioned the interesting experiments of Marthe Vogt I don t know whether you remember that on her suggestion the ratio of oxytocic to vasopressor activity has also been investigated in other species by Schlichtegroll (*Naturwissenschaften* **41** 188 1954) He found that in the cat hypothalamus the ratio was one to one showing that the ratio does vary significantly from species to species

Bargmann The situation is very difficult we used only the dog and we need more investigations on other species

Field In some species the globules which are stainable by the Gomori stain are so very large that it is very difficult to see how they would pass down the axons Also in the dog one finds some nerve cells in the supraoptic nucleus that are so distended that they are virtually signet ring cells

Bargmann They seem to me to be cells in a state of degeneration Occasionally I have seen very small and karyolytic nuclei which are like the vesicles which Verney described as osmoreceptors They are swollen ganglionic cells Concerning the huge droplets mentioned I have not seen such inclusions in the dog but they have been seen in birds I cannot see how these huge particles could move down to the stalk but we must consider the possibility that in some species hormones may be released from the stainable material into the capillaries of the nuclei or that they may be transported to the third ventricle I do not believe we should draw general conclusions from the findings in the dog the cat and the human being The situation in birds seems to be quite different

Fromageot With reference to the material which takes the Gomori stain we have observed that the van Dyke protein both in its active form and free of the oxytocic

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Discussion

Chairman Sir Henry Dale

Dale Might I put one question to Professor Bargmann? Have any experiments been done to show whether water deprivation which robs the neural lobe of its specifically stainable material deprived it only of the antidiuretic vasopressor principle or whether the oxytocic principle goes with it too?

hypothalamic nuclei and I wonder if it is possible that dehydration as well as stress act by stimulation of the adrenal cortex Have you studied the effects of cortisone?

Bargmann We have no experience with cortisone We can see depletion of the neural lobe after total adrenalectomy and you will also see it if you give a rat painful stimuli

Hanstrom There are some points in which my investigations on the mammalian pituitary disagree with those of Professor Bargmann I have found like Professor Bargmann that Gomori stained fibres may end within the pars intermedia but in my experience these fibres are very rare and can probably be regarded as fibres which have gone astray There is another point which must be taken into consideration it is that several mammals have no pars intermedia In whales and elephants there is instead of the pars intermedia a thick membrane of connective tissue between the neural and the distal lobe which must prevent hormones from the posterior lobe from reaching the anterior one The only connexion between these lobes is through the eminentia mediana (infundibulum) and the pars tuberalis by means of the portal vessels

Bargmann I agree entirely with Professor Hanstrom but I said in *some* mammals In the opossum for instance there is a very thin intermediate lobe and you cannot see any connexion with the neural lobe As you know this is a lobulated posterior pituitary and there is no connexion between the two parts but in some species it is very remarkable how the two parts are connected In the golden hamster you can very often see neurosecretory fibres within the intermediary lobe

Rodeck Last year I had the good fortune to spend six months in Professor Bargmann s Institute in Kiel where I was able to study neurosecretion in human foetuses in newborn babies and in young infants Very young infants need a remarkably high quantity of fluid which in a single day may amount to about one sixth or one fifth of their body weight If a grown up person drank the equivalent volume of liquid, he would undoubtedly suffer from diabetes insipidus Hitherto the so called physiological diabetes insipidus of the newborn child or young infant has usually been attributed to immaturity of the kidney The question is whether the regulatory system in the hypothalamus and the neurohypophysis which is responsible for the facultative reabsorption in the distal tubules is fully developed or whether this mechanism matures only gradually during the first year of life as does the kidney We have investigated the whole neurosecretory system (nucleus supraopticus nucleus paraventricularis tractus supraoptico hypophyseus and the neural lobe) in 121 rats 41 guinea pigs 28 dogs and 18 human foetuses newborn infants and children in all stages of life It could be shown that the neurosecretory and hormone producing system is not fully differentiated at the time of birth The majority of the ganglion cells do not show a noticeable development of cytoplasm The neurohypophysis also does not contain neurosecretory material at the time of birth A normal production of neurosecretory material is seen only after several months of post natal life These findings are in accordance with the observations of Heller & Zaimis (*J Physiol* (*Lond*) **109** 162 1949) who found that the neurohypophysis of new born children and young infants contained only a fraction of antidiuretic vasopressor

and vasopressor peptides gives this reaction But we do not know what this fact has to do with the chemical constitution of this molecule

van Dyke I was interested in what Professor Bargmann had to say about the nature of the neurosecretory substance I think that Hild (*Z Anat EntwGesch* **115**, 459, 1951) maintained that this substance is a carrier and that he could extract this carrier and leave the hormones intact In respect of the protein to which Professor Fromageot referred, the only view I hold today is that it may be a storage form of the hormones in the ox gland which I believe is the only one in which it has been investigated I think that it is hazardous to draw conclusions as to whether it is released and in what form the hormones are released A protein like the one isolated by us seems to explain the first experiments of Rosenfeld (*Bull Johns Hopk Hosp* **66**, 398, 1940) who made simple extracts and found that the hormones sedimented as though parts of a protein and that the active materials could be liberated by mild treatment with heat in a weakly acid medium

These findings do support the view that it may be the storage form of the hormone in the ox gland One always has to take into account the possibility of adsorption as pointed out by Sir Henry Dale

Dale This storage form of the hormone gives the vasopressor and the oxytocic actions Does it also give the melanophore expanding action?

van Dyke No, I think that was one of the great pleasures of investigation that the protein containing the activities was virtually free of the hormone affecting melano phores

Croxatto What is the basis for the belief that there are three different substances in the hypothalamus, namely vasopressin adiuretin and oxytocin?

Bargmann When I spoke of three I meant the three different activities tested in the experimental animals The problem whether they should be attributed to three substances is a pharmacological one

Dale Professor van Dyke and other people who have worked with the du Vigneaud pure peptides, tells us that vasopressin and antidiuretin are the same substance, and that oxytocin and the milk ejecting principle are another

Pickford I would like to ask Professor Bargmann s opinion of things that one does see in animals which have had diabetes insipidus for a long time When we stain the hypothalamus afterwards in the areas where the nerve fibres are, we see bunches of black granules rather like bunches of grapes Are these nerve fibres which have pro duced so much material that they cannot pass it on to the posterior lobe and have burst and distributed this material? That is what it looks like

Bargmann To answer this question it would be necessary to see your slides

Noble I believe you said that other forms of stress produce a picture similar to that in which you showed depletion of the gland This brings to mind the finding of the Californian group of workers (Castor Baker, Ingle & Li *Proc Soc exp Biol*, (*N Y*) 76 353 1951) who showed that cortisone apparently produces changes in the

The comparative aspect of neurosecretion with special reference to the hypothalamo-hypophysial system

by

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THE hypothesis that nerve cells produce a secretion with functions which are not associated with the transmission of nervous impulses was for several years regarded as suspect by many biologists However investigations in the first place by E Scharrer in 1928 and later by an ever growing number of scientists has proved without doubt that such neurosecretory material exists and that it differs in several ways from the humoral substances (acetylcholine noradrenaline) which serve the transmission of peripheral nervous impulses It is produced in considerable amounts it can be stained by several histological methods and it is related to hormonal reactions in organs which may be situated far from the site where the neurosecretory material is released into the circulation

WORMS AND MOLLUSCS

Neurosecretory cells have been detected in all groups of animals except the lowest class of invertebrates which possess a nervous system viz the coelenterates But there are some reasons to believe that neurosecretion occurs in as primitive forms as in planarians In segmented worms (polychaetes earthworms) conspicuous signs of neurosecretory activity within numerous cell groups in the brain and the ventral nerve chord have been detected in the shape of vacuoles and brightly staining droplets Some of these cells appear to produce a growth factor which is active in the regeneration of the anterior segments of the body others seem to be concerned with reproduction Extracts from the brain of earthworms increase the frequency of intestinal movements while a decrease of the frequency is caused by extracts from the ventral ganglia The brain of some polychaetes produces an inhibitory hormone which regulates reproduction and prevents a precocious transformation of *Nereis* into the sexually mature form

In molluscs two neuroglandular organs occur in cephalopods the subpeduncular gland and the epistellar body and clusters of neurosecretory cells have been detected in several species of other molluscs (prosobranchs opistobranchs bivalves) However their function is in most cases unknown In some species a relation to the reproductory cycle is suggested and in others a chromatophorotropic action which was shown in species different from that of the donors Moreover the nervous system

and oxytocic activity of the adult Thus, during the time of neonatal development the regulatory system (the neurohypophysis) and the effector organ (kidney) are in wonderfully complete harmony The maturity of the regulatory system corresponds always to that of the effector organ

Heller I am of course very interested in Dr Rodeck s results which agree very well with our findings However I should like to stress that the small amounts of anti diuretic activity that we found in newborn rats and in human infants may not be *insignificant functionally Significance can only be judged in terms of ease of libera* tion and the minimum effective dose on the kidney Even comparatively small amounts stored in the gland of newborns may thus be important

Dale Did Dr Rodeck represent you correctly in saying that the oxytocin content was also very small?

Heller Yes per mg neurohypophysial tissue, the glands of infants contained much less oxytocic activity than those of adults

Sloper *With reference to Dr Rodeck s observations it is of interest that* the newborn seal is far advanced in the matter of hypothalamic neurosecretion The amount of neurosecretory material present in the hypothalamus matches with that of a two month old dog in Dr Rodeck s series

Dale Perhaps the seal has not the common need to concentrate his urine?

Sloper I don t know much about that Sir Can the seal drink sea water?

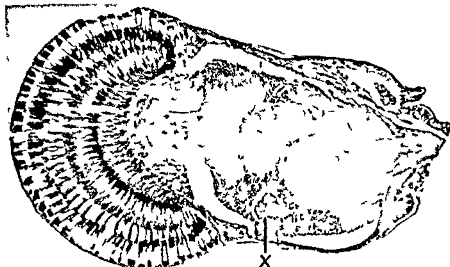


Plate I

Lᴄ ng tudınal section of the eyestalk of a shrımp with the eye to the left the nervous mass ın the centre and the X-organ (Y) ventrally



Plate II

Sac l ke sinus gland of a shrimp Its wall consists ch efly of the swollen nerv erdings of the X cells and other neu secretory cells (see also F g 1)

of *Buccinum* and the hearts of *Buccinum, Pecten* and *Mytilus* contain a cardio inhibitory substance, resembling acetylcholine in its action on the heart of *Buccinum*, and also a cardio excitatory substance whose action on the heart resembles 5 hydroxytryptamine (Welsh, 1956)

In most of these instances of neurosecretory activity in lower invertebrates, the cells which perform this activity, appear to possess the shape of ordinary nerve cells They have dendrites and an axon and form a link in a chain of neurons or innervate effector organs (a muscle or a gland) The neurosecretory product has been observed not only in the cell plasm but also in the axons and migration of the secretory material along the axons has been occasionally demonstrated in polychaetes In addition in some polychaetes (nereids and nephtyids) according to Clark (1956) an axon tract in the brain runs to a connective tissue sheath at its base where it and the pericapsular membrane come to have an intimate connexion with a blood plexus and where the neurosecretory material can be found accumulated on either side of the sheath and around the blood vessels This suggests that in this case the neuro secretory nerve fibres end blindly i e that they do not form a link in a neuron chain but deliver the neurosecretory substance from the axon endings directly into the blood

In a single case in molluscs too Lemche's (1955) observations suggest the presence of the first differentiation of the neurosecretory apparatus into two morphological subdivisions (1) the central neurosecretory cell group, and (2) a separate storage organ This differentiation has been found in all the more closely investigated complex neurohormonal systems in higher invertebrates

CRUSTACEANS

The simplest type of a storage organ in arthropods (if it actually deserves this designation) is the so called neurohaemal organ in crustaceans (Carlisle & Knowles, 1953) which consists of lamellae of neuropile like neurosecretory nerve endings in the pericardium (Alexandrowicz & Carlisle 1953) Some of these nerve endings rise above the inner surface of the pericardium and are supposed to release a cardio accelerating principle (5 hydroxytryptamine) into the blood, while a cardio depressant substance is produced by nerve cells of the trito cerebral commissure Although the nerve cells from which the neurohaemal organs originate are still unidentified their structure ought to be of the same type as those in nereids and nephtyids in which they run to the connective tissue sheath at the base of the brain In crustaceans also these axons terminate blindly Thus they neither innervate any effector organs nor transmit impulses nor form normal links in a neuronal chain

On a still higher level of differentiation is the X organ sinus gland system in crustaceans Here a group of neurosecretory cells constituting the X organ (in a part of the brain which has migrated into the eyestalks) send their axons to a clearly differentiated storage organ, the sinus gland the walls of which consist mainly of the swollen endings of the neurosecretory axons (Plates I and II) Like the X organ the sinus gland in higher crustaceans is situated in the eyestalks where it forms part of the wall of a large blood sinus Recent investigations have shown that in addition to the X organ numerous neurosecretory cell groups within the brain proper send their axons into the sinus gland which thus serves mainly as a multiple storage and release

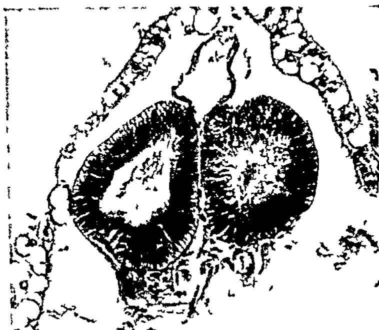


Plate IV

Corpc ra allata n a walking stick insect (*D ppus*) In th s nstance the origina sac like form of the gland s retained

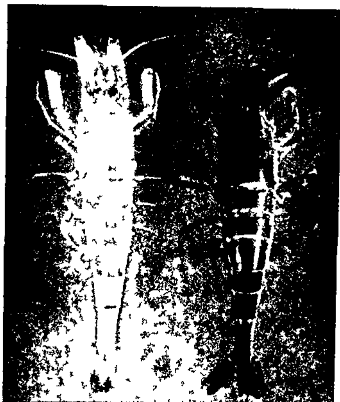


Plate III

To the left an originally dark adapted shrimp (*Cran on*) which 30 minutes previously had been injected with 0 1 ml of a sea water extract of the head of an insect (*Ectobius*) to the right a specimen which 30 minutes previously had been injected with the same amount of pure filtered sea water The latter caused no change of the colour while the extract of the insect head concentrated the p gments of the left specimen so that it became almost pure w h te Both animal were on a dark background

organ for neurosecretory material (Fig. 1). The transport of the neurosecretory substance from its place of origin to its place of storage seems to be by the axoplasmatic current; the speed has been determined in supravital preparations as about 3 mm. per day.

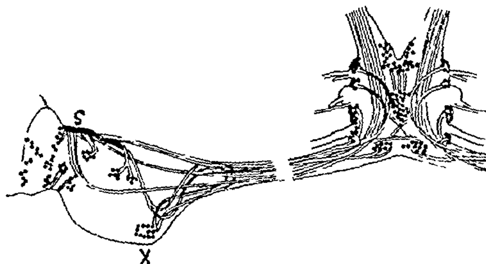


Figure 1

Neurosecretory cell groups and pathways in a crab. The axons of the X-cells (X) and several other clusters are connected with the sinus gland (S). (After Bliss & Welsh 1952.)

The existence of two subdivisions of the neurosecretory apparatus in crustaceans explains several facts of physiological importance. Thus the effects of the substance which is stored in the sinus gland are in part the same as those which can be obtained by extracts from the X organ. Implants of sinus glands into other specimens may be effective at least for a short period, although complete removal of the gland may have a very slight or no effect. In addition, removal of the X organ does not cause an immediate deficiency if the sinus gland with its supply of stored hormones is left intact. Experiments done along these lines have proved that an impressive array of hormonal actions are associated with principles which are stored in the sinus glands of crustaceans and produced by neurosecretory cells within different regions of the nervous mass in the eyestalks and the brain proper. It is probable that different groups and cytological types of neurosecretory cells produce different hormones.

The study of hormonal mechanisms in crustaceans started with investigations on colour change which, according to recent results, appears to be governed by no less than three (perhaps five) principles, one of which probably represents a simple aromatic amine (Östlund Fange 1956). Two other principles regulate the adaptation of the eyes to light and darkness by means of pigment migrations. All these substances are derived from the sinus gland and the central nervous system. The chromatophorotropic hormones are produced in different regions of the central nervous system and accumulate in the sinus gland, extracts and implants of which thus act very strongly on the chromatophores of the skin. In the most beautiful way, and

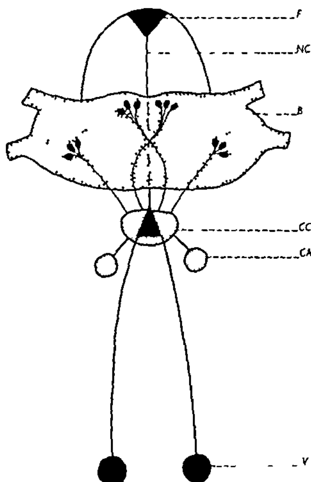


Figure 2

Outline of the brain and the incretory system of the head in insects *F* frontal ganglion *NC* nervus recurrens *B* brain from above with a medial and a lateral group of neurosecretory cells *CC* corpora ca diaca surrounding the hypocerebral ganglion (black) *CA* corpora allata *V* ventricular ganglion

Only a few insects show a physiological colour change which however appears to be controlled by hormones produced by neurosecretory cells in the brain Because of the connexion between these cells and the corpus cardiacum this organ also contains the pigment activating hormone which, by the way has a very strong effect on crustacean chromatophores The slow morphological colour adaptation in locusts however is regulated by a hormone derived from the corpus allatum

The neurosecretory brain cells the corpora cardiaca, corpora allata and the prothoracic gland all partake in the regulation of the complicated morphological and histological changes and the still more complicated co ordination of chemical reactions by means of enzymes which are involved in the metamorphosis of insects

sometimes instantly, the colour is changed to suit the background, according to the intensity of light or to the diurnal period (Plate III)

While only some species of crustaceans show a pronounced physiological colour change, all of them have to shed their hard exoskeleton to be able to grow Thus a still more important hormonal function in these animals is the regulation of moulting and it has been proved that the regulatory substances are neurohormones Most crustaceans from temperate regions exhibit seasonal moultings, the intervals of which are governed by a moult inhibiting hormone produced by the X cells and similar cells in the brain The moult inhibitory hormone is also stored in the sinus gland Lack of this hormone (after extirpation of the X organ or amputation of the eye stalks) is followed by absorption of calcium from the cuticle increase of body water increased oxygen consumption and repeated moulting The hormone appears to be absent in crustaceans which moult throughout the year In a species of shrimp (*Lysmata*) which belongs to this group there occurs on the contrary a moult promoting hormone which is also produced by cells in the brain and the thoracic ganglion, but which is not stored in the sinus gland The primary function of this hormone seems to be the stimulation of a moulting gland proper (the gland of Gabe) which directly starts the preparations for moulting

Like the moult inhibiting hormone another growth inhibiting principle which acts on the ovaries and the testes, is produced by cells of the X organ and the brain In spite of this the hormones do not appear to be identical Hormones from the eye stalks also control the secondary sex characters

In spite of the small size of the X organ and the seemingly simple structure of its cells, it produces still another hormone (or more than one hormone) which controls the calcium phosphorus and water metabolism In the absence of this metabolic hormone the blood sugar concentration decreases The factor which is responsible for the regulation of the water balance appears (at least in *Carcinus*) to be distinct from the principles which control moulting and the development of the sexual glands

INSECTS

In insects also neurosecretory cells are numerous and have important functions They consist of a medial and a lateral group in the brain and of other clusters in the frontal, the suboesophageal and the ventral ganglia The nerves from the brain cells end in the corpora cardiaca which lie in the head in close vicinity to the aorta and beneath the brain From the corpora cardiaca in most insects another nerve runs to two ectodermal glands the corpora allata (Fig 2) Physiologically the neuro secretory cell groups of the insect brain correspond to the X organ in crustaceans and the corpus cardiacum to the sinus gland Like in these organs the neurosecretory material is transported from the brain along the axons into the corpora cardiaca, where it may be stored or released into the circulation In some insects however, a further transport of neurosecretory material along the corpus cardiacum allatum nerve has been observed, and thus the corpus allatum may be directly stimulated by the active principles contained in the neurosecretory material Finally there is in the thorax of insects another endocrine organ the prothoracic gland, which in some respects resembles the moulting gland in crustaceans and which is activated by the neurohormones of the brain

reproductive processes In flies extirpation of the medial brain region which contains one of the neurosecretory cell groups, prevents egg maturation while reimplantation of the same cells promotes it Thus the neurosecretory cells produce a gonad stimulating substance which is stored in the corpora cardiaca Other experiments suggest that the corpora allata also contribute to the regulation of egg maturation and that the principle involved is not identical with that derived from the brain

In certain insects the neurosecretory cells the corpora cardiaca and the corpora allata contribute to the control of metabolism Thus in a species of fly *Calliphora* the absence of the medial neurosecretory brain cells results in a decreased fat and an increased glycogen content in the fat body the corpora allata seem also to be involved in the fat and carbohydrate metabolism and the corpora cardiaca may play a role in the control of the protein metabolism The water balance also appears to be regulated by neurosecretory brain cells but only scattered observations are found in the literature on this subject and no general conclusions can as yet be drawn

Finally the excretory organs in insects the Malpighian tubules show rhythmic movements the frequency of which is stimulated by extracts from the brain and the corpora cardiaca The active principle from the latter organs also increases the amplitudes of the muscle contractions of the Malpighian tubules of the intestine and of the heart The active principle appears to be secreted by specific cells of the corpora cardiaca and to be different from the active neurosecretory substance of the brain Chemically it resembles an orthodiphenol and is thus related to adrenaline

COMPARISONS BETWEEN THE MORE COMPLEX NEUROSECRETORY SYSTEMS OF INVERTEBRATES AND VERTEBRATES

In both crustaceans and insects the principal neurosecretory systems consist of two parts (1) the neurosecretory cell clusters in the brain (several groups in crustaceans two in insects) and (2) a special organ (the sinus gland in crustaceans the corpora cardiaca in insects) in which the neurosecretion is stored The former subdivision corresponds to the nucleus supraopticus and nucleus paraventricularis in the vertebrate hypothalamus and the latter to the neural lobe of the hypophysis (Table 1) Thus the neural lobe represents one of several organs of neurosecretory

TABLE I

The incretory systems of the head in crustaceans insects and vertebrates

	CRUSTACEANS	INSECTS	VERTEBRATES
I	X organ and other neurosecretory cells of the brain	Medial and lateral neurosecretory brain cells	Nucleus preopticus Nucleus supraopticus Nucleus paraventricularis
II	Sinus gland	Corpora cardiaca	Posterior lobe
III		Corpora allata	Anterior lobe

storage and release in the animal kingdom In 1941 when I published the first (but very incomplete) comparison of the neurosecretory systems of insects and vertebrates I was deeply impressed by the occurrence of such similar complicated systems in very distantly related animal groups This seems now still more striking since recent

(Fig 3) During these changes the prothoracotropic hormone from the brain, which is stored in and released from the corpora cardiaca, causes the prothoracic gland to release the so called growth and differentiation hormone This hormone (which was isolated and crystallized by Butenandt & Karlsson in 1954 and found to contain only carbon hydrogen and oxygen in the proportion 4 4 7 3 1 0), is directly responsible for the growth and differentiation which transforms the wingless immature larva (the nymph) into a winged imago with sexual organs In order to prevent a precocious imaginal development the corpora allata during the early life of insects produce a juvenile hormone which counterbalances the hormone of the prothoracic gland The interaction of these two hormones results in the larval moultings during

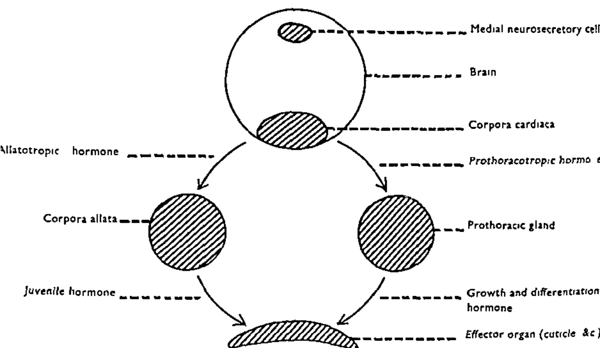


Figure 3

Outline of the nervous and secretory mechanism which controls the moultings in insect

which the animal develops chiefly by growing with very slight signs, if any of adult differentiation Finally at the last larval or nymphal stage the production of the *juvenile hormone* ceases or is considerably decreased and complete metamorphosis takes place It is characteristic of the interaction of the hormones which regulate the post embryonic development in insects that by removal or transplantations of the endocrine glands and by means of parabiosis experiments it is possible to force adults to undergo supernumerary moultings and become giants to cause larvae or nymphs to undergo a precocious differentiation and to become dwarfs and finally by supplying an excessive amount of juvenile hormone to cause adult tissues to reassume a juvenile shape

In insects as in crustaceans *neurosecretory hormones* play an important role in

paraventricularis of higher vertebrates are presumed to be derived from the nucleus preopticus in lower vertebrates one must consider the possibility that they also may have different functions

A third and very striking fact which derives from the study of the neurosecretory cells in polychaetes, crustaceans and insects is the domination of neurosecretory function in these animals Thus in *Nepthys* three quarters of the brain consist of cells which exhibit signs of a neurosecretory activity (Clark, 1955) and all the better investigated hormonal phenomena in crustaceans and insects are governed by neurohormones The nervous system in these animals is evidently as much concerned with the regulation of body functions by the slower hormonal processes as by the more speedy nervous mechanisms Thus the close similarities between the architecture of the neurosecretory systems in invertebrates and vertebrates almost force one to suspect that there must be more numerous neurosecretory mechanisms in vertebrates than the production of the posterior lobe hormones

It is now about ten years since I worked on neurosecretion in invertebrates and since I turned to similar studies in vertebrates On this occasion therefore I should have liked to report some new results of investigations of the pituitary and the neurosecretory system in mammals The results of current work on the neurosecretory pathways in hibernating mammals such as the hedgehog (*Erinaceus europaeus*) during the winter and in mammals from hot deserts such as the kangaroo rat (*Dipodomys merriami*) are, however, not quite complete Although living under very different conditions both these animals have to economize body water The kangaroo rat and related forms live in the hot dry regions of the south western U S A one of these species occurs even in the Death Valley, the deepest point of which is 84 m below sea level A maximum air temperature of +56 C has been recorded in this area for a long time a world record According to K & B Schmidt Nielsen (1952) the kangaroo rat is able to live and thrive on dry plants without any access to drinking water and has survived for years in the laboratory when fed on dry grain only It does not store an excess of water in the body and it has no exceptional resistance to dehydration It obtains water by oxidation of its food and expends it with the greatest economy The extrarenal water loss of the kangaroo rat is only about half of that of the white rat and its urine may be about twice as concentrated Kangaroo rats which are forced to drink water excrete it with great difficulty and are liable to water intoxication (Hofmann, 1956) The unphysiological situation was indicated by the occurrence of haematuria in more than half of the animals in the experiment Ames & van Dyke (1950) have investigated the amounts of antidiuretic hormone in the urine and the posterior lobe of kangaroo rats They found up to 50 mU of antidiuretic hormone in the urine as against about 6 mU /ml in dogs after powerful osmotic stimulation and about the same in laboratory rats deprived of water for 48–72 hours The posterior lobe in kangaroo rats contained 0 9 mU of antidiuretic hormone per microgramme against only about 0 3 mU in normal laboratory rats Moreover the neural lobe of *Dipodomys* weighs relatively more Thus although laboratory rats are 5 to 6 times larger than kangaroo rats their neural lobes are only 3 times the size of those of kangaroo rats and their pituitary contains less antidiuretic hormone

When I examined the neurosecretory system in *Dipodomys* I did not find any features which are essentially different from those in laboratory rats as described

investigations have shown that similar neurosecretory systems exist in chilopodes diplopodes and arachnoids (Gabe, 1955) The only instance in which a homology between these systems could be suspected is in the myriapodes and insects But be this as it may, it is clear that complex neurosecretory systems have appeared independently at least four and perhaps six times in the animal kingdom

While all the neurosecretory systems reviewed here show the two subdivisions mentioned, a third one has to be added in insects and vertebrates viz the corpora allata and the glandular lobe of the hypophysis respectively This represents a further analogy these organs are both of ectodermal origin, the corpora allata being pouch like invaginations from the ventral side of the head (Plate IV) while the glandular lobe originally forms a similar sac like invagination from the embryonic stomodæum, i e Rathke s pouch Thus in both insects and vertebrates the principal neurosecretory systems consist of a nervous component, which forms the first two subdivisions, and an ectodermal component, forming the third subdivision

The functional relations between the neural and the glandular lobe of the pituitary are still keenly debated but taken together, the hormones released by the pituitary cover about the same functions as the neurosecretory system in insects namely colour change growth reproduction metabolism and water balance The array of hormones produced by the main incretory organs of insects (and crustaceans) would therefore seem to be hardly less complex than that of vertebrates including man Histologically the neurosecretory cells of the crustacean X organ and those of the insect brain may indeed be regarded as of a more specialized type than most of the cells of the nucleus supraopticus and paraventricularis in vertebrates I have already pointed out that the neurosecretory cells of the lower invertebrates thus far described seem to have the shape of normal nerve cells (with dendrites and an axon) which form a link in a neuron chain or innervate an effector organ Most cells of the nucleus supraopticus and paraventricularis have dendrites but the corresponding cells in crustaceans and insects are unipolar and have no dendrites or collaterals Like the neurosecretory cells of the hypothalamus they end blindly and do not form links in a neuron chain in so far as they do not innervate any effectors Morphologically, they are reduced to a simple thread like transport apparatus for the carriage of the neurosecretory substance from the place of production to the place of storage

This account of neurosecretion in invertebrates has of necessity been brief and many important details had to be omitted Our knowledge of invertebrate endocrinology has very much increased since I published my monograph on the subject (Hanstrom, 1939) I therefore recommend the more recent reviews by Ernst & Berta Scharrer (1954*a* *b*) Gabe (1954) Berta Scharrer, (1953 1955) and Arvy Gabe & B Scharrer (1956), and concerning the comparison between the neurosecretory systems of invertebrates and vertebrates the papers of Hanstrom (1941 1954) and E & B Scharrer (1944)

Another conclusion resulting from this comparison is connected with the fact that in insects the neurosecretory brain cells consist of two groups and in crustaceans of several There are some reasons to presume that the two cell groups in insects have different functions and very strong arguments have been presented in favour of the view that in crustaceans different clusters and cytological types of neurosecretory cells produce different hormones Although the nucleus supraopticus and nucleus

in his book of 1742 *The brain considered anatomically physiologically and philosophically* In this treatise he declared that the soul has established in the cerebrum an illustrious chymical laboratory the products of which he considered were separated and filtered in the infundibulum finally to trickle down to the hypophysis The chymical operation, he continued may be called not only a secretion and purification but also a rectification and alcoholization May I be permitted to add that Swedenborg was hardly thinking of alcohol in the present meaning of the word but evidently used it as a synonym for spirit and evanescent essence

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by Hillarp (1949) Ortmann (1951), Eichner (1953), Wagenvoort (1954) and Imai (1954) It is possible that a laborious count of the number of cells in the hypothalamic neurosecretory nuclei may give a clue to the high concentration of the antidiuretic hormone in the neural lobe of the kangaroo rat Preliminary examination in several species of rodents of the size of the neural lobe, relative to that of the glandular lobe indicates that in general the neural lobe in desert and hibernating species is relatively larger than in other rodents from temperate regions (Enemar & Hanstrom, 1956)

Herring and Cushing among others assumed that the posterior lobe hormones derived from cells of the pars intermedia which invaded the processus infundibuli This hypothesis was still maintained by Buchner (1950) and Romieu, Stahl & Occelli (1952) perhaps because these authors considered the human hypophysis only in which such an invasion is a normal phenomenon However, in other mammals which I examined during comparative studies of the pituitary (Hanstrom, 1952) I often found no invasion of intermedia elements into the posterior lobe and in the desert rodents investigated it was not found at all

Bucy, Romeis and the school of Ranson regarded the pituicytes as the source of the posterior lobe hormones The role of these cells is as yet not quite clear but the best founded hypothesis is undoubtedly that formulated by E Scharrer and W Bargmann who assume a neurosecretory origin for the neurohypophysial hormones It is of interest in this connexion that in crustaceans and insects also the water balance is regulated by neurosecretory hormones The concept of the origin of the posterior lobe hormones from invading intermedia cells or from pituicytes made it necessary for the protagonists of this view to assume migration of the posterior lobe hormones from the pituitary gland towards the hypothalamus from which considerable amounts of the hormones have been extracted The conception of neurosecretion postulates movement of the neurosecretory substance and the hormones in the opposite direction, i e from the hypothalamus to the neural lobe This process however, is only yet another instance of the truth of the proverb nothing new under the sun For as Zuckerman (1954) has pointed out, Andreas Vesalius wrote in his work *De humani corporis fabrica libri septum* published 400 years ago (1555) that the infundibulum was a funnel through which the phlegm from the brain trickled down into the pituitary (Fig 4) The Swede Emanuel Swedenborg expressed the same opinion

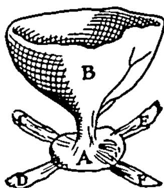


Figure 4

Vesalius s conception of the funnel (infundibulum) (*B*) through which the phlegm from the brain trickles down into the pituitary (*A*) The four imaginary ducts (*C D E F*) carry the phlegm from the gland (After Zuckerman 1954)

stomach tube once an hour day and night for 10 days Serial sections through the hypothalamus were stained by Gomori s method simultaneously with sections from a 'control animal that had been maintained on a similar diet Comparison of the sections from the two animals showed the following features In the anterior division of the supraoptic nucleus the cells of the hydrated animal were markedly paler than the control with an apparent deficiency of neurosecretory material In the posterior division of the supraoptic nucleus the differences were less marked, but a higher proportion of pale cells were exhibited by the experimental animals than the controls The cells of the paraventricular nucleus showed the greatest change In the hydrated animal these cells were uniformly pale and almost completely devoid of neurosecretory material Conversely the descending tracts from the paraventricular nuclei of the hydrated animal were characterized by heavy accumulations of stainable material in the form of stained axons and clumps which extended throughout the dorso ventral extent of the hypothalamus The appearances of the posterior lobes of the two animals did not differ in any marked way Changes seen in a second hydrated animal were in the same sense but were less marked This second animal had become somewhat lethargic during the experiment whereas the first hydrated animal had remained perfectly happy

Dale What was the effect on the specifically staining substance in the posterior lobe itself?

Jewell There was no detectable difference in the posterior lobes

Dale I am trying to get a picture of what this staining material is doing It is increased in the nerve cells and it has got into the tracts but apparently it hasn t yet reached the lobe or is it being poured out of the lobe as fast as it gets there?

Jewell I would be very interested to hear Professor Bargmann's comments

Bargmann I wonder if these accumulations of stainable material are the same as Dr Pickford has seen

Pickford Yes very similar

Bargmann These are lobulated Herring bodies and they seem to be a sign of storage in the course of the axon Under normal conditions we can see the same thing in birds Possibly you saw some ruptures on your slides perhaps due to physiological or technical conditions You can show that these Herring bodies are part of the axon

Hanstrom We have found such lobulated or star shaped structures several times in mammals for instance in wolves and sometimes they appear to be connected to one proximal and another distal fibre In some instances I think that they may constitute nerve endings and in some respects resemble the bulb shaped endings which I have described in a number of mammals These endings are very common and outstanding in some specimens

Dale What species are you speaking about?

Hanstrom The tiger

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Discussion

Chairman Sir H Dale

Jewell There is a question I would like to ask Professor Hanstrom about the first part of his talk Have experiments been done to exchange the hormones from insects to crustaceans or vice versa?

Hanstrom Yes especially the colour change hormones When one makes an extract of the corpus cardiacum of insects and injects this extract into shrimps they change their colour as completely as they would if they had been injected with their own colour change hormone and the hormone from the insect corpus cardiacum is some times even more potent than the true shrimp hormone

Dale There was a letter recently in *Nature* (Carlisle & Butler **177**, 276 1956) which described an extraordinarily interesting observation, that the ovary inhibiting hormone from the eyestalk of the prawn is effective also in bees producing sterile workers And it was also stated that a substance from the Queen Bee will keep a female prawn from ovulating

Jewell Dr B Andersson and I have made some observations on the neurosecretory material of dogs hydrated for a period of days We gave a dog 200 ml of water by

amount of this material, it implies that the paraventricular nucleus is particularly concerned with the process Yet there are observations of Olivecrona (*Nature*, (*Lond*) 173, 1001, 1954) which suggest that the paraventricular nucleus is particularly concerned with oxytocin production So this is still very much in the air

Harris, G H There is one question I would like to ask Professor Hanstrom, and that is whether he had done anything to alter the activity of the anterior lobe and whether he had noticed any difference in the neurosecretory material I am thinking particularly of ovariectomy increasing the production of gonadotrophic hormone and whether there is then any difference in the neurosecretory material in the median eminence

Hanstrom No, I am sorry, we haven t done that

Martini I wanted to ask Professor Bargmann what he thinks about Barnett s method which stains disulphide groups and thus seems to reveal directly the oxytocic and antidiuretic hormones in hypothalamic neurons Are the results obtained by Gomori s and by Barnett s methods comparable?

Bargmann I am very careful concerning histological and histochemical questions I said only yesterday to Professor Heller that while we are able to stain the tracts electively we are not able to make any conclusions about the chemical nature of the substances stained I know Dr Sloper has another opinion—perhaps he would say something because he is an expert on these matters

Sloper Professor Bargmann has asked me to discuss the applicability of Barnett s thioglycollate DDD reaction for cystine to the study of neurosecretion This reaction is difficult to perform I learnt it from Professor Montagna and like him find it useful when applied to skin Perhaps because of its sensitivity it is far less useful in the hypothalamus because it has not the selectivity for neurosecretion of the Gomori techniques On the other hand the thioglycollate ferric ferricyanide and performic acid Alcian blue reactions introduced by Adams and myself demonstrate structures rich in cystine and are if anything more selective than the Gomori techniques With these techniques we demonstrate a protein rich in cystine in the hypothalamus and neurohypophysis It diminishes after dehydration and accumulates above the cut pituitary stalk I do not think it is for me as a pathologist to say more concerning its relationship to posterior pituitary principles

Jewell Did you notice a lot of this material in the tiger?

Hanstrom Yes

Jewell Has Professor Hanstrom any suggestion to offer to explain the remarkable development of stainable material in the carnivores? The dog and cat, and apparently the tiger, show large quantities Dr Sloper mentioned earlier the large amounts to be seen in the seal I have examined the supraoptic and paraventricular nuclear cells of the grey seal, using Gomori s method, and the intensity of staining in these cells is similar to the dog In the desert rat on the other hand, in conformity with Professor Hanstrom s observations on his desert animals, I found no unusual amount of material

Hanstrom I have also found lobulated Herring bodies in a marsupial and in a tree shrew It is not only carnivores I think

Lloyd Would you estimate that there is more of this neurosecretory material in the hypothalami of these animals which you hydrated? The reason I ask is that if you hydrate a rat giving it nothing but liquid for three or four days you will find that the content of antidiuretic material in the hypothalamus is actually less than the normal If you hydrate it acutely there appears to be more It looks as though the supply is keeping up with the demand

Jewell If you estimated the amount in the cell bodies in the hydrated dog there would certainly be less but since there is great accumulation in the axons in the lateral hypothalamus the total amount in the hypothalamus may still be high

Martini Dr Kovacs has observed that hydration produces in rats a drop of neuro secretory material, both in the supraoptic and paraventricular nuclei and in the hypothalamo hypophysial tract

Lloyd One has to dehydrate animals severely by giving them a hypertonic solution to cut down on the hypothalamic antidiuretic activity

Sloper Dr Jewell s observations on the loss of neurosecretory material from the para ventricular neurones of the hydrated dog are of the greatest interest I would like first to make a plea for great caution in the interpretation of these changes Varia tions in staining intensity and section thickness can be most deceptive although of course the changes in Dr Jewell s sections were clear cut I would secondly like to ask Dr Jewell whether all the paraventricular neurones were similarly affected I ask because I have been making photometric estimations of histochemically demon strated neurosecretory material in the hypothalamus of the dehydrated rat Although most neurones show depletion a number are full of neurosecretion Has Dr Jewell seen such neurones in the hydrated dog and, if so how does he account for their occurrence?

Jewell There was uniform depletion of the neurons in the paraventricular nucleus whereas in the supraoptic nucleus there were often neurons persisting which were quite as full as in the normal animal This was a very curious observation I thought for if there is a real correlation between the state of hydration of the animal and the

The relationship of oxytocin and vasopressin to active proteins of posterior pituitary origin

Studies concerning the existence or non existence of a single neurohypophysial hormone

by

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FOUR activities of extracts of the bovine posterior hypophysis have been known for a long time these are the uterus stimulating (oxytocic) activity the milk ejecting (galactogogic) activity the blood pressure raising action (vasopressor activity), and the antidiuretic activity *A priori* one might think that these four activities correspond to four different substances Since 1920 there have been two opposing views, one held by Abel and his co workers (Abel Rouiller & Geiling 1923 Abel 1930) according to which the oxytocic vasopressor and antidiuretic properties belong to a single substance and that of Dudley (1919) who considered that the oxytocic and vasopressor activities were due to different substances The first conception led MacArthur (1931) and later van Dyke and his co workers (van Dyke Chow Greep & Rothen 1942) to isolate a pure protein which possessed the four activities mentioned The second concept led Kamm (Kamm Aldrich, Grote Rowe & Bugbee 1928) and later du Vigneaud (1952) and his team to purify the different active principles In the course of this work two peptides were obtained in pure form the one—oxytocin—possessing oxytocic and galactogogic properties the other—vasopressin—possessing the vasopressor and antidiuretic activities The problem therefore was whether one or two substances are normally responsible for the activities in posterior pituitary extracts i e whether the hormone is a protein of which the two peptides oxytocin and vasopressin are degradation products which retain the activities or whether these peptides normally exist in the gland, and are used independently by the organism in accordance with its needs The concept of the existence of a single hormone rests on a physiological as well as on a chemical basis the ratio of vasopressin to oxytocin activity (V/O) in the neurohypophysis of several mammals is unity when the activities are determined by reference to the International Standard (Waring & Landgrebe 1950) Moreover there is simultaneous liberation of the oxytocic and antidiuretic (or vasopressor) activities on electrical (Harris, 1947), osmotic (Abrahams & Pickford 1954) or chemical stimulation (Chamorro & Minz 1955)

It appeared desirable to us to study the question of the existence of a single hormone using both the chemical and the physiological approach In the chemical

* Read by C Fromageot

activities and amino acid composition of the two peptides Thus, electrodialysis permits separation of oxytocin and vasopressin from inert protein material without hydrolysis

Precipitation with trichloracetic acid

A 1 per cent solution of active protein was precipitated by 5 per cent trichloracetic acid 50 per cent of the oxytocic activity 70 per cent of the vasopressin activity and 8 per cent of the initial nitrogen were found in the supernatant solution After removal of the acid by electrodialysis the active substances in the cathode solution were studied Here again electrophoresis on paper revealed only the two substances which behaved like pure oxytocin and vasopressin A preparative electrophoresis was carried out and the paper was cut into sections of 2 cm These were eluted with water and the activities of the eluates were determined No oxytocic activity was found except at the point corresponding to oxytocin and no vasopressor activity was found anywhere except at the point corresponding to vasopressin (Fig 1) Moreover

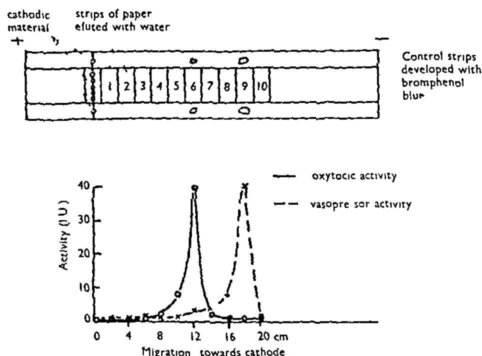


Figure 1

Paper electrophoresis of the cathodic material obtained after electrodialysis of the supernatant resulting from the precipitation of active protein with 5 per cent trichloracetic acid

hydrolysis of the substances yielded the amino acids corresponding to the composition of oxytocin and vasopressin Thus trichloracetic acid precipitates the protein while leaving the major part of the oxytocic and vasopressin activities in solution

investigation we have subjected the protein of van Dyke (ox gland extract) to different purifying procedures to determine whether the oxytocic and vasopressor activities always remain with the protein In the physiological investigation we have studied the changes in the *V/O* ratio of the neurohypophysis of the rat during growth and reproduction

RELATIONSHIP BETWEEN OXYTOCIN, VASOPRESSIN AND THE PROTEIN OF VAN DYKE

The active protein was prepared according to van Dyke *et al* (1942) A powder having oxytocic or vasopressor activity of 18–20 I U per mg was obtained The nitrogen content and the sedimentation and diffusion constants corresponded to the values given by the American authors We have subjected the active protein to various procedures of purification which did not involve the possibility of hydrolytic cleavage

Dialysis and ultrafiltration

On dialysis against water all the activity and nitrogen remained inside the sac After ultrafiltration of most of the solution through a cellophane membrane (oscillating ultrafiltration technique of Ambard) neither nitrogen nor biological activity was found in the ultrafiltrate Therefore neither dialysis against water nor ultrafiltration permit the separation of active substances of low molecular weight from the protein of van Dyke On the other hand when a solution of 50 mg of the protein in 0 2 N acetic acid was dialysed against 80 volumes of acetic acid (0 2 N, pH 2 75) at room temperature, all the oxytocic and vasopressor activity was found outside the bag, although 88 per cent of the initial nitrogen was recovered from inside showing that mere dialysis against dilute acetic acid produces dissociation

Electrolysis and electrodialysis

The active protein dissolved in acetate buffer at pH 4 4 was subjected to electrophoresis in the Tiselius apparatus After 4 5 hours, the solutions in the anode cathode and central compartments were analysed for nitrogen and vasopressor activity The activity of the initial solution per mg of nitrogen was equivalent to that of 125 I U of vasopressin After electrophoresis the solutions in the anode central and cathode compartments had vasopressor activities equivalent to 44, 150 and 190 I U per mg N respectively As no inactivation occurred the heterogeneity of the material was obvious the principle responsible for the vasopressor activity was more rapidly displaced towards the cathode than the other constituents

Fifty mg of the active protein were dissolved in water with the assistance of a trace of acetic acid and placed into the central compartment of an electrodialysis cell consisting of three compartments The anode and cathode compartments contained water Cellophane membranes were used A potential of 900–1,000 volts was applied and precautions were taken to assure that the temperature of the solution did not rise above 25° C After 9–11 hours, 90–100 per cent of the initial oxytocic and vasopressor activities (about 1,000 I U) were recovered in the cathode compartment but only 7–8 per cent of the initial nitrogen The cathode solution was concentrated and studied by electrophoresis on paper It was possible to demonstrate two substances only these migrated like pure oxytocin and pure vasopressin and had the

have detected no inactivation after 5 hours treatment at room temperature Further, Lawler & du Vigneaud (1935) have shown that trypsin inactivates vasopressin but not oxytocin We have hydrolysed the active protein with trypsin (enzyme substrate ratio 1 50, pH 7 8, 38 C) After 6 hours the vasopressor activity of the protein had completely disappeared but the oxytocic activity was quite unaffected

Taken as a whole it is evident from these experiments that the vasopressor and oxytocic activities of the active protein behave towards chemical and enzymatic agents exactly like the vasopressor and oxytocic activities of vasopressin and oxytocin On the other hand processes which do not involve hydrolysis such as dialysis against dilute acetic acid, electrodialysis precipitation by trichloracetic acid and counter-current distribution dissociate the active protein into substances responsible for the oxytocic and vasopressor activities and at least one inert protein The complex, which is broken neither by dialysis against water nor ultrafiltration nor ultra centrifugation (Rosenfeld, 1940 van Dyke *et al* 1942) may be an artefact of the ex traction process or may possess biological significance In the latter case it appeared of interest to determine whether the complex behaved in the same manner during the course of the synthesis of the active principles and their utilization by the organism

VARIATIONS IN OXYTOCIC AND VASOPRESSOR ACTIVITIES DURING GROWTH AND LACTATION

Several authors (Simon 1934 Waring & Landgrebe 1950 Dicker & Tyler 1953*b*) have found that the *V*/*O* ratio is approximately unity in the neurohypophysis of the adult rat Although Dicker & Tyler (1953*b*) found a ratio of 1 the absolute values of the two activities were 50 per cent less than those of Simon (1934) On the other hand Kuschinsky & Liebert (1939) found a ratio of about 2 (these authors deter mined the antidiuretic activity which corresponds to the vasopressor activity New determinations seemed therefore necessary Adult albino rats of 220 ± 30 g were used which were obtained from four different colonies The animals were killed by decapitation The hypophysis was immediately removed and allowed to stand in pure cold acetone several hours The posterior lobe was then separated, homogenized with 0 5 ml of 0 25 per cent acetic acid and heated at 100 C for 1 5 minutes The volume was adjusted to 1 ml with water and the material assayed for vasopressor activity by the method of Landgrebe Macaulay & Waring (1946) and for oxytocic activity by the method of Holton (1948) The determinations were made at different times of the year The results obtained are shown in Table 1

The following conclusions may be drawn

(1) the activities are very constant (0 8 unit per gland) (2) the ratio of vasopressor activity to oxytocic activity is about 1 (3) there is no difference between the two sexes

These results are in excellent agreement with those of Simon (1934) Several authors have determined the antidiuretic activity which is a property of vasopressin and have found values around 0 8–1 0 unit per gland (Heller 1947 Cavallero Dova & Rossi 1954 Morel 1954) The agreement is therefore very good

A ratio of vasopressor (or antidiuretic) to oxytocic activity of approximately 1 has also been found in man cat dog sheep pig and horse (Heller & Zaimis 1949 Waring & Landgrebe 1950 Dicker & Tyler 1953*a* *b*) This ratio holds generally for

Counter current distribution

Twenty mg of the active protein were subjected to counter current distribution in the system secondary butanol – 0 5 per cent trichloracetic acid After 24 transfers, the contents of each tube were homogenized by the addition of water and the biological activities and the nitrogen content were determined It was found that the oxytocic and vasopressor activities were separated from one another and from the principal nitrogen containing substance (Fig 2) The active substances were isolated

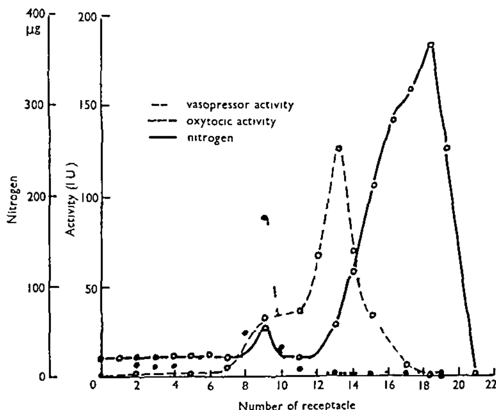


Figure 2

Counter-current distribution of the active protein in the system secondary butanol—0 5 per cent trichloracetic acid

Subjected to paper chromatography they behaved like oxytocin and vasopressin Moreover vasopressin and oxytocin subjected to counter current distribution in the system described had the same partition coefficient as the principles obtained from the active protein

Action of chemical agents and enzymes

Sealock & du Vigneaud (1935) reported that oxytocin and vasopressin were not inactivated by reductions with cysteine We have reduced the active protein with cysteine (30 mg of cysteine hydrochloride per 0 15 mg of nitrogen at pH 7 8) and

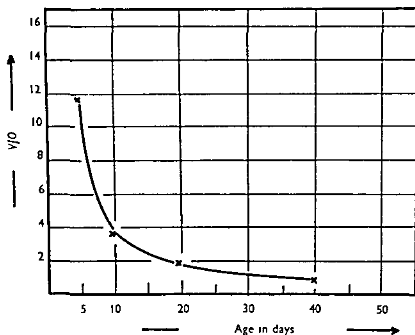


Figure 3

Ratio of vasopressor to oxytocic activity (*V/O*) in the pituitary glands of infant rats

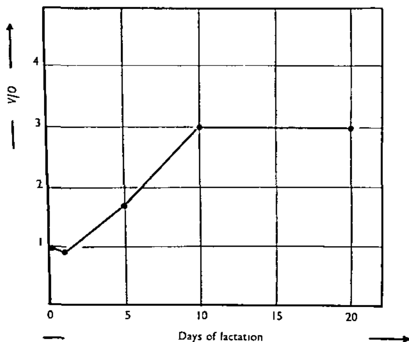


Figure 4

Ratio of vasopressor to oxytocic activity (*V/O*) in the neurohypophysis of lactating rats

mammals though not in the whale, guinea pig and rabbit (Waring & Landgrebe 1950, Dicker & Tyler 1953*b*, Schlichtegroll, 1954).

We have studied the change in the *V/O* ratio in the neurohypophysis of the rat during growth and lactation.

TABLE I

Content of oxytocic and vasopressor activity in the neurohypophysis of the adult rat

V = Vasopressor activity in milliunits per gland
O = Oxytocic activity in milliunits per gland

Female			Male		
V	*O*	*V/O*	*V*	*O*	*V/O*
800	850	0.94	825	825	1.00
800	850	0.94	800	840	0.95
825	825	1.00	800	820	0.97
800	840	0.97	825	840	0.98
820	810	1.01	810	850	0.95
800	850	0.94	800	820	0.97
Mean 804	837	0.97	810	832	0.97

Variation in the ratio of vasopressor to oxytocic activity during growth

In the case of 5 day old animals 10–15 glands were pooled for extraction. 5–10 glands were used of 10–20 day old rats and in the case of older animals a single gland. The hypophyses of 5 day old rats were extracted without removal of the anterior lobe. Figure 3 shows the variation in the *V/O* ratio as a function of age.

Our results indicate that the *V/O* ratio reaches unity during growth. An identical shift has been found by Dicker & Tyler (1953*a*, *b*) in the dog and in man during embryonic development. Moreover these authors have observed that the *V/O* ratio was higher in young than in adult rats, cats and guinea pigs. It appears therefore that the synthesis of the substance with vasopressor activity is not synchronized with that of the oxytocic substance. It is possible that the second principle is only of importance in reproduction and for this reason is synthesized somewhat later.

Variation in the ratio of vasopressor to oxytocic activity during lactation

Five groups of rats were used: animals which were killed 2–3 hours, 12–24 hours, 5 days, 10 days and 20 days after parturition. Rats of the first group, unlike those of the others, had not yet commenced to lactate. Figure 4 shows the variation in the *V/O* ratio as a function of the stage of lactation.

The ratio, which is unity in normal animals, is approximately 1 at the time of parturition and during the subsequent 24 hours but it increases during lactation due to a decrease in the amounts of the oxytocic principle.

following 24 hours This decrease does not occur if one prevents the young from suckling It appears therefore that the neurosecretory material, if it actually corresponds to a chemical substance and not to a non specific physical configuration (Scharrer & Scharrer 1954) decreases at the beginning of lactation while the hormone content increases However, Hild & Zetler (1953) have reported that maceration of the glands in a mixture of alcohol and chloroform eliminated the chromogenic material of the neurohypophysis without causing loss of the activities

Our experiments as a whole demonstrate on the one hand that it is not possible to extract from the neurohypophysis a chemically pure substance endowed with both vasopressor and oxytocic activity and on the other that under normal physiological conditions the organism synthesizes and utilizes the vasopressor substance and the substance with oxytocic activity independently Thus it appears that vasopressin and oxytocin are true hormones Further investigations are necessary to determine whether the complex formed by vasopressin oxytocin and inert protein is an artefact of extraction or whether it has biological significance

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Figure 5 shows the levels of vasopressor and oxytocic activity in the neurohypophyses of the animals in the five groups

We found that the levels of the vasopressor and oxytocic activities were about 50 per cent below normal at the time of parturition but that they returned to normal 12–24 hours later then, the oxytocic activity alone diminishes The last observation is in agreement with the results of Dicker & Tyler (1953*b*)

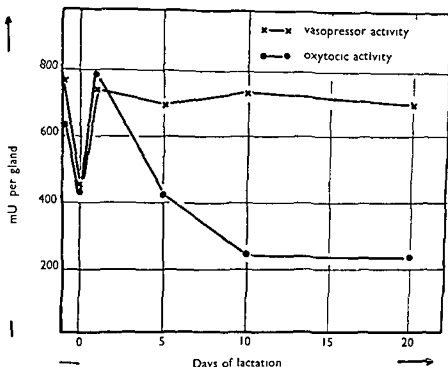


Figure 5

Vasopressor (×——×) and oxytocic (●——●) activity in the neurohypophyses of lactating rats

The first effect of suckling is the re establishment of the levels of the neurohypophysial hormones to their normal value during the first 24 hours This was shown as follows The rats were separated from their offsprings 2–3 hours after parturition and killed 12–24 hours or 10 days afterwards After 12–24 hours the levels of the two activities in the glands were only slightly higher than those observed at the time of parturition they had not become normal as in lactating animals After 10 days the levels had slightly increased but had only reached 70–80 per cent of the normal Suckling which produces an increase in vasopressor and oxytocic activity during the first day following parturition acts inversely on the level of the substance in the neurohypophysis which is stained by the Gomori reaction as modified by Bargmann (1950) Stutinsky (1953) observed in rats that this material which is present in approximately normal quantity at parturition diminishes considerably during the

ratios in non pregnant female rats of the same strain which in our hands at least show a considerable variation I have taken statistical advice on this and I am informed that in the small groups so far available, there is no clear difference between the *mean* values for the controls and the lactating animals I think perhaps that to see more clearly we should follow the suggestion of Professor van Dyke and assay oxytocin by a milk ejection method as well as by uterine response, and vasopressin by antidiuretic assay as well as on the blood pressure We are in the process of doing so

Fromageot I quite agree with Profes or Heller that one must be careful about methods of assay many factors may indeed interfere with the titrations such as the presence of substances with potentiating or inhibitory effects the physiological state of the animals or of the organs used &c but care has been taken to make the assays as correct as possible and in the curves which I showed each point is the average of six different experiments The variations within each of the series were significantly less than the differences observed between the groups of animals

Croxatto Professor Fromageot what is the amino acid composition of the inactive protein molecule after the separation of the active peptides Is it different from vaso pressin and oxytocin?

Fromageot The amino acid composition of the inactive protein obtained after separa tion of the active peptides is not very different from that of the active protein as a whole because of the small weight of the peptides compared to that of the protein Without remembering what this amino acid composition is, I may say that apart from its richness in cystine the protein in question contains much proline A preliminary analysis has been made by Block & van Dyke (*Arch Biochem* 36 1 1952) and it differs quite clearly from the composition of each of the peptides I might add that the molecular weight of the active protein is about 28 000 The molecular weight of each active peptide is about 1000 Therefore what remains after this removal does not differ significantly from the initial substance in respect also of its molecular weight

Landgrebe I was very interested in Professor Fromageot s results with lactating rats and since van Dyke s protein comes from ox glands I should like to tell you our results in the ox We have tested glands from heifers, milking cows and bulls at various times of the year and found that there was amazing uniformity in the amount of activity in the glands There is no difference between the milking cow the heifer or the bull in the amount of activity or in the ratio

Mrs Dodd We have investigated this problem in goats in relation to lactation We tried milking them out immediately before killing and removing the gland The glands were removed in from 90 seconds to 4 minutes and there was no difference in the vasopressin oxytocin ratio and again not very much difference in the total amount weight for weight of dry gland tissue between lactating goats and non lactating goats

Ginsburg I think this is very interesting because I do not think it has been appreciated how rapidly the gland can be repleted Professor Fromageot showed that there was

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Discussion

Chairman R Gaunt

Heller I should like to make a plea for not relying too much on the amounts of oxytocic or vasopressor activity found in the glands of any particular strain of rat If one looks through the literature one finds that some workers have found as much as 800 mU per 100 g rat and others only 250 mU 100 g We originally found high values, but a year later with another strain we obtained values that were very much lower As to the hormone ratio I think it is certainly, as I have shown, with many others of the order of 1 in the adult rat but again I do not think one should interpret that too precisely After all there are two biological assays involved in these estimations and both of them have very considerable errors One cannot therefore say more than that the value is somewhere near 1 but in some animals it may be 1 3 or even 1 5 Whether this is a real difference I cannot say With the Chairman s permission I would like to show a slide of the vasopressin oxytocin ratios in the neural lobe of lactating rats as determined by Mr K Lederis in my laboratory We have used rats from 1 to 21 days after parturition The methods of assay were very much the same as those used by Fromageot and his co workers, and the results at first glance appear similar to Professor Fromageot s curve On the first and third day a ratio of something like unity was found, thereafter it rises but these values must be compared with the

Polypeptides with posterior pituitary-like activities

by

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HYDROLYSIS of blood serum or of some of its proteins by certain proteolytic enzymes liberates polypeptides which have many of the pharmacological actions of the neuro hypophysial hormones

On one hand it has been reported that the digestion of either blood serum or hypertensinogen with trypsin (or with snake venoms) produces a substance called bradykinin (Rocha e Silva 1951) which has a pronounced vasodepressor effect and a motor action upon the plain muscles of the hollow viscera, including the uterus On the other hydrolysis of these substrates with pepsin sets free several peptides including a hypertensin like substance viz pepsitensin (Croxatto & Croxatto 1942) Some of these peptides have a motor effect upon uterine muscle and at least one of them has an inhibitory effect upon urinary excretion (Croxatto Rojas & Barnafi 1951) This antidiuretic substance has been provisionally named pepsanurin It attracted our attention because although the uterotonic activity is shared by many peptides e g hypertensin pepsitensin bradykinin kallidin (Werle 1955) pepsitocin and substance *P* (von Euler 1936) no peptide resembling vasopressin has so far been found in tissues other than the hypothalamus or the hypophysis

Since oxytocin and vasopressin are simple octapeptides (du Vigneaud Ressler & Trippet 1953 du Vigneaud Lawler & Popenoe 1953) and a larger molecule may be endowed with the same activities (van Dyke Chow Greep & Rothen 1942) it does not seem strange that polypeptides set free from other substrates show some of the same pharmacological effects Hypertensin according to a recent paper of Skeggs Marsh Kahn & Shumway (1955) contains 8 amino acids in approximately unimolecular proportion plus two moles of histidine Seven of these amino acids are also contained in either oxytocin or vasopressin The purest preparations of hyper tensin so far obtained besides producing a powerful vasopressor effect have intense oxytocic activity and are also capable of modifying diuresis and natriuresis (Pickering & Prinzmetal 1940)

I would like to take this opportunity to describe some polypeptides which are liberated from hypertensinogen as well as from other fractions of blood serum by pepsin and which show the activities of neurohypophysial hormones Since these substances have not yet been purified some of the results given here must obviously be regarded as preliminary

rapid repletion of hormones in the glands of rats suckled immediately after parturition Perhaps in Mrs Dodd's experiments although the gland was depleted very quickly it was also repleted very quickly and the time interval between killing the animals might have been too long If repletion rate is rapid and variable this will render the estimation of hormone content of glands an unreliable index of secretion

Mrs Dodd We took it that the total amount of hormone which would be released from the gland by the natural stimulus of the let down of milk would be so minute in relation to the total amount of substance there that one would not be able to demonstrate it

Herdan Professor Heller s experiments on statistical analysis show that there is a trend in the vasopressin oxytocin ratio but its probable error is of the same order of magnitude as the trend itself and hence it fails to be significant The mean values for vasopressin oxytocin ratios in lactating rats and control rats were 1 138 and 1 147 respectively with a probable error of 0 075 Taking twice the standard error as the borderline between significance and non significance, one concludes that, firstly, the ratio is not significantly different from unity, and secondly that there is no significant difference between the average vasopressin oxytocin ratios for the lactating and the control group

These results are given with the proviso that they refer to small numbers of experiments (20 in the lactating and 9 in the control group) Since the standard error is inversely dependent on the sample size it is quite possible that greater samples may show significant differences

THE PROPERTIES OF THE ANTIDIURETIC SUBSTANCE (PEPSANURIN)

Pepsanurin is soluble in formic acid, acetic acid and in 20 per cent trichloroacetic acid, insoluble in benzene, petrol ether, ether pyridine and acetone and scarcely soluble in absolute alcohol, concentrated N butanol and secondary butanol It can be precipitated from aqueous solutions by the addition of 10 volumes of acetone Charcoal amberlite (IR 50) and permutite (pH 4 5) adsorb it It can be eluted from charcoal by glacial acetic acid in which it keeps its activity indefinitely It can be separated from glacial acetic acid by a mixture of absolute ethanol and petrol ether (1 1) or with purified ether It can be eluted from permutite with a solution containing 1 per cent acetic acid and 5 per cent NaCl In contrast to vasopressin, pepsanurin is partially extracted by secondary butanol equilibrated with 0 05 per cent acetic acid It dialyses through cellophane membranes It is destroyed by alkali but withstands heating in organic acids Performic acid as employed by Popenoe & du Vigneaud (1953) to oxydize vasopressin (a procedure that leads to its inactivation) also destroys the activity of pepsanurin containing extracts

The antidiuretic effect can be obtained in rats by injecting the extract in different ways intraperitoneally subcutaneously or intravenously The effect is longer lasting and greater after intraperitoneal than after subcutaneous injection

The inhibition of diuresis in water loaded rats (conscious or in ethanol anaesthesia) is not the result of delayed water absorption from the intestine at the end of an experiment the amount of water in the digestive tract is slightly higher than in non injected controls but it is about the same as that found in rats injected with vasopressin Since the injection of pepsanurin containing extracts into hypophysectomized rats with diabetes insipidus produces a profound inhibition of diuresis the antidiuretic effect cannot be explained by stimulation of the neuro hypophysis and release of vasopressin Polyuria in such rats can be counteracted by an adequate dose of the extract administered at intervals from 6 to 12 hours The inhibition of diuresis can also be obtained in water loaded hypophysectomized rats which have been treated with cortisone (Fig 2) Both pepsanurin and vasopressin can completely inhibit the polyuria produced by renin Both substances inhibit more markedly when injected 60 to 90 minutes after renin

In the white rat pepsanurin as well as vasopressin can antagonize the increased diuresis and natriuresis produced by 25 to 50 mU of purified oxytocin containing less than 3 per cent of vasopressin (Fig 3)

Sympatholytic drugs such as dibenamine or hydrogenated ergot alkaloids which produce a powerful inhibition of the antidiuretic effects of enteramine (Erspamer 1952) do not modify the antidiuresis caused by either vasopressin or pepsanurin

The antidiuretic effect of pepsanurin seems to be due mainly to an increase of water absorption by the renal tubules However a vasoconstrictor action upon the glomerular vessels cannot be excluded although there is no systemic hypertensive effect Moderate amounts of pepsanurin (providing they do not decrease the output of urine below 50 per cent of that of the controls) do not have a significant effect upon creatinine clearance but if there is an intense inhibition of diuresis creatinine clearance is strongly affected It can be assumed from this, that in addition to increased tubular reabsorption of water a decrease in glomerular filtration may be

It was established first of all that by treating hypertensinogen with pepsin, a vaso pressor substance (which we called 'pepsitensin') could be obtained Besides having various chemical properties in common with hypertensin, pepsitensin acts in a similar way upon several types of plain muscle Like hypertensin it is inactivated by all the crystalline proteolytic enzymes including pepsin After pepsitensin has been formed by the incubation of hypertensinogen with pepsin (at pH 3 5 for 3 to 4 hours) and the protein has been discarded, the residual extract contains the vasopressor principle, mixed with large amounts of inactive peptides If this is subjected to further digestion with pepsin (at pH 2 5) it loses all of its vasopressor effects and a great deal of its oxytocic activity but an antidiuretic activity appears The same result can be obtained, if instead of the two successive digestions, hydrolysis is performed in one stage for a longer period (8–14 hours)

The antidiuretic substance does not necessarily derive from pepsitensin if hypertensinogen preparations are treated with renin long enough to convert the hyper tensinogen entirely to hypertensin which is then dialysed away, treatment with pepsin does not then cause liberation of pepsitensin Instead, it gives rise to a potent antidiuretic substance Sera of different mammals have been used as starting material (human ox cat and rat) with similar qualitative results Hydrolysis of human and ox serum albumin by pepsin also sets free substances with a marked antidiuretic action After purification involving adsorption with charcoal elution with glacial acetic acid and fractional precipitation with acetone and ether an extract is ob tained containing 30 per cent of the initial activity 0 2 *ml of this extract, contain* ing 1 to 3 mg of solids produces an antidiuretic effect which lasts up to 2 to 3 hours after injection (Fig 1)

ANTIDIURETIC ACTIVITY OF PEPSANURIN (PN) (GILMAN GOODMAN TEST)

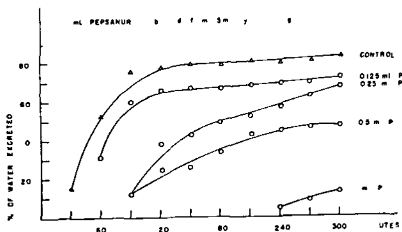


Figure 1

Antid uretic effect of different doses of an extract from horse serum hypertensinogen

produced The latter effect has also been noticed when a pronounced antidiuretic effect is obtained with vasopressin (Barnafi Croxatto & Krause 1956)

The profound influence that neurohypophysial extracts exert upon the water balance of the amphibians can also be observed with digests of hypertensinogen or of plasma hydrolysates In *Bufo chilensis* the latter preparations increase the water uptake through the skin, i e they produce a similar effect as that caused by posterior lobe extracts in frogs (Brunn 1921)

Persanurin seems to be a peptide since chymotrypsin completely destroys its activity (Figs 4 and 5) which neither pepsin nor carboxypeptidase are able to do, trypsin has only a slight effect Vasopressin shows a surprisingly similar behaviour carboxypeptidase and pepsin have no effect and chymotrypsin destroys it completely trypsin quickly destroys the vasopressor activity but the antidiuretic activity of vasopressin is only partially affected

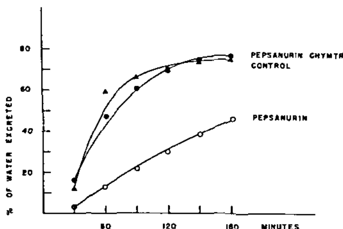


Figure 4

Antidiuret c effect (Burn s test) of 0 2 ml of an extract contain ng pepsan urin and of 0 2 ml of the same extract incubated with chymotrypsin The p eparation was obtained f om hors serum hypertensinogen

Pepsanurin does not seem to originate from a specific substrate in plasma it has been obtained from both serum albumin and serum globulins when using human plasma fractioned by the method of Green & Bumpus (1954) Albumin yielded a slightly higher antidiuretic activity than hypertensinogen but most of the experiments described up to date were carried out with extracts from hypertensinogen from different sources

Using purified pig hypertensinogen* an antidiuretic substance is released even after removal of the hypertensin formed by previous incubation with renin When this preparation is injected no vasoconstrictor effect is shown but a slight and

* A highly purified sample generously suppl ed by M F Bumpus

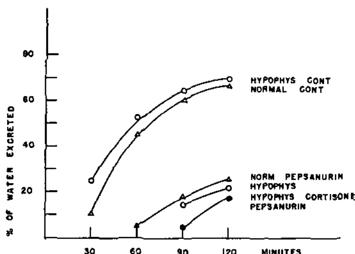


Figure 2

Percentage urine output of water loaded rats (water load 5 per cent of body weight)

(1) Hypophys Cont = Hypophysectomized rats injected wi h o 5 ml of o 9 per cent NaCl solution and previously treated w th cortisone

(2) Normal Cont = Normal rats injected with o 5 ml of o 9 p r cent NaCl solution

(3) *Norm Pepsanurin = Normal rats injected with o 5 ml pepsanurin* (horse serum hypertensinogen) extract

(4) Hypophys Pepsanurin = Hypophysectomized rats injected with o 5 ml pepsanurin extract

(5) Hypophys Cortisone Pepsanur n = Hypophy ectomized rats previously treated with cort sone and njected with o 5 ml pepsanurin extract

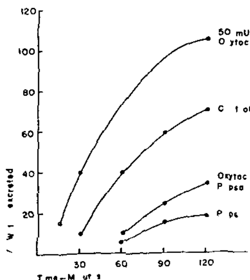


Figure 3

Effects up n diuresis obtained in water loaded rats with injections of

(*a*) 50 mU oxytocin subcutaneously

(*b*) o 9 per cent NaCl solution subcutaneously

(*c*) 50 nU oxytocin s bcutaneously plus o 1 ml p psanu in extract intrap riton eally

(*d*) o 1 ml p psanur n extract int ap r ton eally

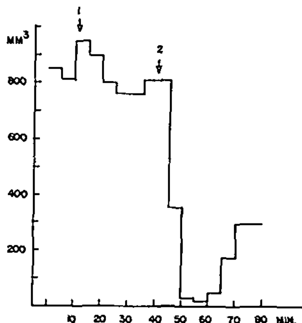


Figure 5

Rat anaesthetized with ethanol Urine collected continuously and the volume measured every 5 min

1 = Injection of 0 1 ml of an extract obtained from hypertensinogen It was treated with renin dialysed and incubated for 3 hours with chymotrypsin

2 = Injection of the same preparation not incubated with chymotrypsin

transient fall of blood pressure is seen From 5 to 10 mg hypertensinogen an extract is finally obtained with an antidiuretic potency equivalent to that of about 10 mU vasopressin (Venezian 1955) Pepsanurin containing extracts have oxytocic effects of varying intensity No extract free of oxytocic activity has as yet been prepared

The antidiuretic principle differs from vasopressin since it has no pressor activity It is inactivated by chymotrypsin but the serum of pregnant women does not affect it (Fig 6) although it destroys vasopressin very easily (Croxatto Barnafi & Ferrer 1954*b*) This suggests a difference in the chemical composition of the two principles

PEPSITOCIN

As reported before it seems possible that an oxytocic substance different from the antidiuretic principle occurs in peptic hydrolysates Thus extracts with the same antidiuretic activity may depending on their origin have a different oxytocic potency A higher yield of antidiuretic substance is obtained from the albumin fraction of plasma, while higher oxytocic potency is obtained from the globulin fraction The oxytocic activity withstands the action of pepsin and trypsin but it is destroyed by chymotrypsin and is more easily extracted from water by butanol than is pepsanurin

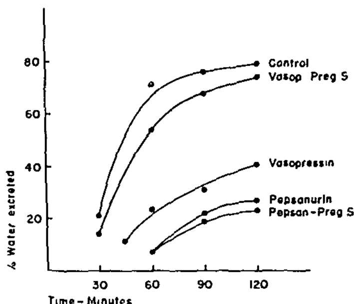


Figure 6

Antidiuretic effects in rats (Burn s test) injected with vasopressin and pepsanurin both incubated and non incubated with the serum of pregnant women (eighth month)

Control rats injected with 0 9 per cent NaCl solution

Vasop Preg S = 0 mU vasopressin incubated for 6 hours with 0 15 ml pregnancy serum

Vasopressin – 30 mU vasopressin

Pepsanurin = 0 1 ml extract obtained from human serum

Pepsan Preg S – 0 1 ml of the same extract incubated for 6 hours with *0 15 ml pregnancy serum*

The oxytocic potency of hypertensinogen extracts is very slowly destroyed when incubated at pH 7 to 7 2 with carboxypeptidase this does not occur with oxytocin

It can be assumed that pepsitocin is different from bradykinin or pepsitensin (Venezian 1955) When hypertensinogen is previously treated with renin so as to exhaust hypertensin production and hypertensin is then eliminated by dialysis tryptic action does not produce bradykinin (Rocha e Silva 1951) nor is pepsitensin produced if digestion is accomplished by pepsin (pH 3 5) after prolonged treatment with renin If hypertensinogen preparations free of hypertensin are submitted to a long period of digestion with pepsin (pH 2 5) an extract having both oxytocic and antidiuretic activity and with no vasoconstrictor activity can be prepared

Hypertensinogen hydrolysates previously treated with renin show less oxytocic activity than untreated extracts The contrary is observed with the antidiuretic potency When hypertensinogen is hydrolysed with pepsin for 11 hours and the alcohol soluble polypeptides (pH 5 2) are removed the denatured hypertensinogen remaining forms neither an oxytocic nor an antidiuretic substance if submitted to a second hydrolysis with pepsin (Venezian 1955)

The purification of both pepsitocin and pepsanurin is still in a preliminary stage

and it would therefore be premature to assume a relationship between them and the active peptides of the neurohypophysis or the albumin isolated by van Dyke, Chow, Greep & Rothen (1942) However, it can be claimed that they are not identical pepsanurin differs from both vasopressin and van Dyke's protein because it has no vasoconstrictor effect and resists the action of the vasopressinase contained in the serum of pregnant women Pepsitocin also is not inactivated by pregnancy serum and it differs further from oxytocin because it is destroyed by carboxypeptidase, suggesting that it has free carboxyl groups

GENERAL REMARKS

The substances released by the hydrolytic action of pepsin might be regarded as artefacts of pharmacological interest only But if pepsin is able to liberate hypertensin like substances from the same substrate as that for renin one is tempted to consider the possibility that the blood proteins could also supply precursors for other peptides Specific enzymes in the tissues or in the blood might react with circulating substrates to produce other active peptides It is possible for example that hypothalamic nuclei and the hypophysis are capable of elaborating active peptides by hydrolytic mechanisms

On the other hand the presence of an antidiuretic substance among the peptides formed by the enzymatic action of pepsin could explain some controversial results of estimations of antidiuretic activity in blood the antidiuretic effects of serum plasma or blood when injected intraperitoneally into water loaded rats, are due not only to the antidiuretic hormone but also to a different substance presumably similar to pepsanurin In our experience the antidiuretic activity of blood when measured with Burn s (1931) test is always higher than expected In other words it is greater than could be accounted for by the amount of vasopressin contained in the blood and released by neurohypophysis under different conditions This high antidiuretic potency *is not due to some of the factors developed during coagulation (Croxatto Pfister* & Munizaga 1955) It diminishes or disappears after incubation at 38 C or after prolonged dialysis (Croxatto Andrade & Barnafi 1952) Recent experiments demonstrated that mere acidification (to pH 4) of plasma increases both antidiuretic and oxytocic potency whether the plasma had been dialysed for a few hours or not We could show that the level of antidiuretic and oxytocic activity of the blood in hypophysectomized rats decreases slightly during the first week following the operation but that it is then maintained even when the blood is obtained from rats during a period of polyuria

When estimated by intraperitoneal injections both the oxytocic and the antidiuretic activity were as high in the blood of patients with diabetes insipidus as in normal subjects (*Croxatto, Barnafi Lopez & Andrade 1954a*) (*Fig 7*) Moreover we have found an antidiuretic substance in the blood of pregnant women which must be different from vasopressin since as is well known (von Fekete 1930 Page 1946 Croxatto Reyes & Croxatto 1947 Croxatto Vera & Barnafi 1953) during pregnancy blood inactivates the antidiuretic hormone at a high rate Nevertheless during the last month of pregnancy the antidiuretic potency of plasma as measured by the intraperitoneal method is much higher than that in the plasma of non pregnant women All this leads to the assumption that a protein substrate in the

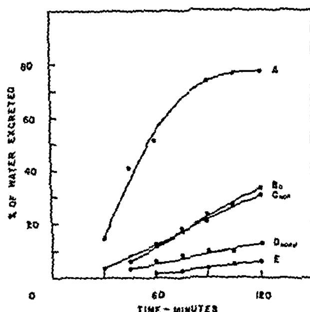


Figure 7

Percentage of water excreted by water loaded rats (Burn's test) Water administered simultaneously with intraperitoneal injection A = 1 ml of 0 9 per cent NaCl solution mean of 15 experiments each performed on a group of 4 rats B = 0 5 ml per 100 g body weight of serum of 5 patients with diabetes insipidus mean of 7 experiments C = 0 5 ml of serum of normal subjects per 100 g mean of 7 experiments D = 1 ml of serum of normal subject per 100 g mean of 8 experiments E = 1 ml per 100 g of serum of 5 patients with diabetes insipidus mean of 8 experiments

plasma which to some extent is protected from inactivating factors gives rise to the substance which produces this antidiuretic effect It is liberated by dialysis and incubation at 37 destroys it When plasma is injected into the intraperitoneal cavity of the rat the active substance is liberated and the antidiuretic effect is produced

When serum or plasma is deprived of its antidiuretic activity either by incubation or by prolonged dialysis and is then treated with pepsin the antidiuretic action becomes even stronger than it was initially Thus it would seem that there are at least three antidiuretic factors in the plasma firstly the neurohypophysial antidiuretic hormone which can be rapidly removed by dialysis and which is easily inactivated by vasopressinase secondly a substance linked with proteins and protected from the action of inactivating factor but which can be fairly well eliminated by dialysis or which can be destroyed by prolonged incubation and thirdly a factor which is liberated by the hydrolytic action of pepsin The third factor is the substance for which we have provisionally coined the name pepsanurin

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Discussion

Chairman R Gaunt

Gaunt Are all your substances active intravenously?

Croxatto Yes they are active However the antidiuretic substance differs from vasopressin in being less active when given intravenously

Noble The findings presented showing a marked reduction in the total output of urine in the rat after the injection of active fractions raise the question of specificity of such action in the Burn method of assay Although I would like to discuss this matter in more detail in my own paper we have felt that in the assay of urine extracts the test rats should excrete at least 50 per cent of the administered water If they excrete less we believe that antidiuretic values obtained are erroneous, and that non specific toxicity or local vascular effects may be responsible for the antidiuretic action Assays at different dose levels would be of interest and I wonder if Dr Croxatto has run tests at other than the single dose level indicated on his slides?

Croxatto Our antidiuretic assays were not confined to that described by Burn and we found an antidiuretic effect with all methods Neither toxic effects nor circulatory changes were found even when the substances were injected intravenously We got the same results with preparations of varying degrees of purity

Gross Did you notice any hypotensive activity with your peptide? It is well known that certain peptides extracted from the plasma and also from the hypothalamus have a long lasting hypotensive activity

Croxatto In rats these extracts have no effect upon blood pressure except when given in very large amounts They were not tested in cats

van Dyke Do these preparations have any effect on the renal circulation i e on renal plasma flow and glomerular filtration rate?

Croxatto We have estimated creatinine clearance and we find the same effect as that obtained with vasopressin with moderate doses there is no effect

van Dyke I doubt if anyone has really shown that vasopressin can be detected in peripheral blood of intact animals the amount present is so minute that it can hardly be estimated accurately Mirsky (Mirsky Stein & Paulisch *Endocrinology* 54 491 1954) for instance finds the high figure of about 20 mU 100 ml in rats His control experiments were done in dogs and would suggest extremely high levels of hormone

The other factor I would like to comment on is the alleged diuretic effect of oxytocin We have studied purified synthetic oxytocin and the natural product and we have invariably obtained an antidiuretic effect Fraser (*J Physiol (Lond)* 101 236 1942) detected no diuretic effect after very large intravenous doses in dogs

Croxatto When blood is injected intraperitoneally we are quite certain that the effects seen are not solely due to vasopressin Something else is contained in or liberated from the blood which increases the antidiuretic effect This may explain why we have

found no differences in the antidiuretic potency between the blood of normal subjects and patients with diabetes insipidus

Concerning the diuretic and natriuretic effects obtained with our oxytocin preparation and the different results obtained with pure oxytocin, we can assume either that the former contain a contaminant substance or that we are dealing with species differences In white rats natriuretic effects with oxytocin were always obtained but this was not so in grey rats Possibly this difference can be attributed to the fact that grey rats being very excitable animals, respond to the injection with a release of vasopressin

van Dyke My remarks, of course apply to highly purified oxytocin and not to your preparations

Toffey I wonder if the different results after intraperitoneal and intravenous injections are partly explainable by lymphatic absorption A protein injected into the peritoneum would be absorbed by lymphatics and we know that lymph glands can do strange things to proteins in a matter of 10 minutes or so It might be worth while to see what happens when you incubate some of your preparations with lymphatic tissue

Croxatto We thought that perhaps the liver was involved but your suggestion requires consideration

Walker Are the activities of pepsitocin and pepsanurin retained after incubation with sodium thioglycollate, which is a substance often used by some of us to try to identify substances of pituitary origin?

Croxatto This has not been tested

Heller With regard to the effect of oxytocin I believe that Professor van Dyke did not get a natriuretic effect after injecting it intravenously into dogs I wonder if there may be a species difference we have confirmed the reports of a natriuretic effect of oxytocin in the rat and it seems possible that this is due to the renal vascular changes which can be produced by Pitocin in this species (Dicker & Heller *J Physiol (Lond)* **104**, 353 1946) but so far nobody seems to have reported an increase of renal sodium excretion in the dog

van Dyke This is a possible explanation

Pickford We have sometimes found oxytocin to cause a sodium outpouring in the dog but this is not always so and may depend upon the size of the dose we used

Sawyer Whether one gets a diuretic effect with oxytocin in the rat seems to depend upon the sodium load (Sawyer *Amer J Physiol* 1952 **169**, 583) If the rat is excreting a large amount of sodium oxytocin may increase this and thus produce an osmotic diuresis The diet, therefore might be important in determining whether one sees a diuretic response or not

In Dr Croxatto s experiments in which he showed the antidiuretic effect of whole serum injected intraperitoneally, did he exclude the possibility that the dialysable antidiuretic substance is 5 hydroxytryptamine rather than a polypeptide?

Croxatto This is eliminated because the effect was not blocked by dibenamine and hydrogenated ergot alkaloids

Eisen Oxytocic activity is very readily produced in blood, plasma or serum from many mammalian species including man Professor C A Keele and his collaborators have shown that such activity may result from mere contact with glass (Armstrong, Keele, Jepsen & Stewart, *Nature* (*Lond*) **174**, 791, 1954) This uterine stimulation is ascribed to a substance provisionally called pain producing substance (PPS) It differs from 5 hydroxytryptamine in several aspects, and from bradykinin in its susceptibility to proteolytic enzymes I have tested these substances in rats anaesthetized with ethanol and I found that their antidiuretic action is very variable when injected intravenously For example, 5 hydroxytryptamine rarely produces a reduction in the urine flow in doses smaller than 5–10 μg /kg , often 20 μg /kg or more are needed When it is found that an antidiuretic substance is more potent by the subcutaneous than by the intravenous route, one should bear in mind that there are several ways in which the urine flow may be reduced such as pain stress and non specific toxicity I wonder also whether Professor Croxatto would have obtained oxytocic activity with less drastic extraction procedures

Croxatto Chymotrypsin destroys the oxytocic and antidiuretic activity of my extracts which eliminates the possibility that it is due either to 5 hydroxytryptamine or to other non peptide substances We have found that simple treatment of plasma with hydrochloric acid (pH4) gives rise to oxytocic and antidiuretic activity

Eisen With regard to the specificity of this antidiuretic effect, I have noted that pain will produce an antidiuretic response in an animal with established diabetes insipidus following section of the supraoptico hypophysial tract

*The storage and liberation of neurohypophysial hormones**

by

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IN all mammals the principal site of storage of the neurohypophysial hormones is in the posterior lobe of the pituitary body. Recently, as a result of the observations of Ranson and his co-workers, Verney, Harris and other investigators on the hypothalamic control of the liberation of the hormones as well as a renewed interest in the secretory activity of certain hypothalamic neurones (E. and B. Scharrer, Bargmann and their collaborators), the distribution of the hormones in the hypothalamus has been carefully scrutinized, especially in the dog.

Early experiments suggested that differences among species may be considerable. Abel (1924) was perhaps the first to report that simple extracts of the hypothalamus contain vasopressor and uterine-stimulating substances. Abel used tissues of sheep. Later van Dyke (1926) could detect only a minute amount of oxytocin in the tuber cinereum of the ox. In the same tissue of the dog Sato (1928) and Trendelenburg (1928) found more oxytocin than in the bovine tuber cinereum as well as a considerable amount of antidiuretic hormone; the quantities of both hormones in the tuber cinereum increased sharply after hypophysectomy. All subsequent work indicates that the hypothalamus of the dog contains more antidiuretic hormone than that of any other mammal. The information concerning other mammals is much less complete and includes observations, often fragmentary, on man, the macaque monkey, ox, camel, hog and rat. Table 1 summarizes these reports which are based upon assay methods of varied reliability. The best established figures are believed to be those for the dog. The proportion of total vasopressor-antidiuretic hormone in the hypothalamus is about 7 to 50 times as great in the dog as in other animals. A strikingly smaller proportion of the total oxytocin is in the canine hypothalamus which in this respect, although relatively rich in the hormone, resembles that of other mammals.

Peculiarities of the storage of the hormones in gross divisions of the hypothalamus are of interest. These are illustrated in Fig. 1 by a comparison between the dog and the camel based upon assays of the dissected tissues in our laboratory (van Dyke, Adamsons & Engel, 1955; Adamsons, Engel, van Dyke, Schmidt-Nielsen & Schmidt-Nielsen, 1956). All divisions of the hypothalamus of the dog were much

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TABLE I

*The distribution of neurohypophysial hormones in the hypothalamus and posterior lobe of the pituitary**

Animal	Vasopressin		Oxytocin	
	Hypothal	Post lobe	Hypothal	Post lobe
	per cent	per cent	per cent	per cent
Dog	20 0	80	2 3	> 97
Dog[a]	15 0	85	8 0	92
Camel	0 4	> 99	1 2	> 98
Ox[a]	0 2	> 99	0 3	> 99
Hog[a]	0 5	> 99	0 5	> 99
Rat[b]	3 0	97	< 1 0	> 99
Monkey (Macaque)	0 4	> 99	—[c]	> 99
Man	0 7	> 99[d]	—[c]	> 99[d]
Man[a]	1 7	> 98	2 5	> 97

a Hild & Zetler 1951 or 1952
b S Henry unpublished
c Less than 50 mU 6 700 mU in posterior lobe of monkey
d Two specimens includes infundibular stem

Some results of Hild & Zetler are used in Table 1 The reliability of their methods of assay and the validity of their conclusion that vasopressin and antidiuretic hormone are different substances have not been accepted by van Dyke Adamsons & Engel (1955) Their data alone are available for the hypothalamus of the ox and hog their figures for vasopressin and antidiuretic hormone have been averaged on the assumption that the same substance had been assayed by two different methods

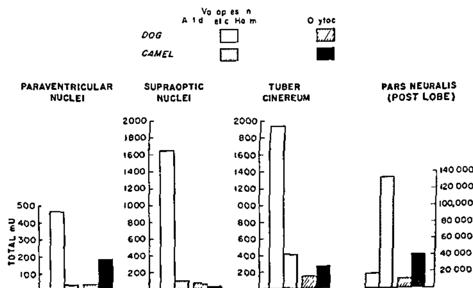


Figure 1

The distribution of vasopressin and oxytocin in the neurohypophysis of the dog and camel (Data of van Dyke *et al* (1955) and of Adamsons *et al* (1956))

richer in vasopressin whereas, at least in the paraventricular nuclei, the camel tissue contained more oxytocin The difference in the amount of oxytocin in the tuber cinereum of the dog and camel was not significant The total amount of both hormones was of course, much greater in the camel neurohypophysis owing to the much greater size of the posterior lobe (about 65 mg after desiccation by acetone in comparison with 3 5 mg for the dog)

The ratio of vasopressin to oxytocin in terms of the stored products in the hypothalamus and posterior lobe has an important bearing on theories concerning the secretion of the polypeptides Information concerning the ratio of the two principles in several mammals is summarized in Table II comparisons of the ratio in divisions

TABLE II

Approximate ratios (V/O) of vasopressin to oxytocin in the hypothalamus and posterior lobe

Animal	V/O in	
	Hypothal	Post lobe
Dog	17	1 5
Camel	1 1	3 3
Rat	5	1 3

V/O in the posterior lobe of the ox hog monkey and man (3 specimens) were 1 4 1 1 1 5 and 1 2 respectively

TABLE III

Approximate ratios of vasopressin to oxytocin in divisions of the hypothalamus and posterior lobe

Animal	V/O in			
	Paraventric nuclei	Supraoptic nuclei	Tuber cinereum	Posterior lobe
Dog	15	24	14	1 5
Camel	0 2	2 2	1 5	3 3

of the hypothalamus of the dog and camel will be found in Table III The reader is warned that the ratios listed represent approximations especially in the rat hypothalamus which contains only about 16 mU of vasopressin and 3 4 mU of oxytocin as average amounts

According to the theory of neurosecretion, the neurones of the paraventricular and supraoptic nuclei secrete the hormones which migrate in or along their axones and are finally stored in the posterior lobe It is said that a carrier substance, different from the hormones, serves for transport and is identified histologically by the chrome alum haematoxylin phloxine stain (Hild & Zetler 1953) Among the animals listed in Table II the dog clearly differs from the other mammals in the very high ratio of vasopressin to oxytocin in the hypothalamus, 17, in comparison with the low ratio of 1 5 in the posterior lobe (Vogt, 1953, reported *V/O* ratios of approximately 14 in the hypothalamus and of 1 in the posterior lobe of the dog) No explanation of this discrepancy has been suggested by experiments Miss Sarah Henry, working with canine or murine hypothalami and posterior lobes incubated the tissues in various media but was unable to demonstrate a formation of either hormone or a conversion of one to the other *in vitro* Great technical difficulties were encountered, especially in exact dissection Vogt (1953) suggested that the site of elaboration of oxytocin might be in the posterior lobe or that its final synthesis might occur during its transfer in the axones terminating in the posterior lobe Another possibility is that different neurones secrete vasopressin and oxytocin and that usually except for vasopressin in the dog, final synthesis takes place in the posterior lobe Lastly it is conceivable that the transfer from the hypothalamus to the posterior lobe may be very rapid for oxytocin and slow for vasopressin in the dog whereas it is relatively rapid for both hormones in other mammals

A comparison of the *V/O* ratios in the dog and camel in which estimates are based upon identical methods of assay is given in Table III The very low ratio (0 2) in the paraventricular nuclei of the camel in comparison with about 15 in the dog suggests a high rate of secretion of oxytocin in these nuclei in the camel This finding may be related to Olivecrona s (1954) conclusion based upon briefly described experiments, that the same nuclei in the rat regulate the production of oxytocin by the posterior lobe The *V/O* ratio in the posterior lobe of the camel pituitary although clearly different from the dog and ox is not exceptional Ratios of 2 to 3 have been reported in the posterior lobe of the guinea pig rabbit, wallaby and finback whale and of 12 5 in the sperm whale Even higher ratios have been found in foetal posterior lobes (For references see van Dyke Adamsons & Engel 1955)

The investigations which have been made furnish no information concerning the rate of secretion of the hormones by hypothalamic neurones A high rate of secretion could be associated with a rapid transfer to the posterior lobe and a low concentration in hypothalamic nuclei Possibly a slow rate of transfer accounts for the exceptionally large quantity of vasopressor antidiuretic hormone found in the dog s hypothalamus If the quantity of hormone in the hypothalamus be taken as 1 the stored product in the posterior lobe is 4 for vasopressor antidiuretic hormone and about 44 for oxytocin in the dog In the camel the corresponding figures are about 225 and 80 respectively Other mammals appear to resemble the camel rather than the dog Presumably the hormones found in the tuber cinereum represent secretions undergoing transfer to the posterior lobe

The principal depot for storage is the posterior lobe In only one mammal the ox is there information concerning stored hormones Rosenfeld (1940), showed that the oxytocic and pressor activities of fluid pressed from posterior lobes behaved in the

ultracentrifuge as if parts of one or more large molecules, after the fluid had been heated at a pH of about 4, the activities appeared to be small molecules sedimenting at a very much slower rate A protein which could account for Rosenfeld s findings was isolated by van Dyke, Chow Greep & Rothen (1942) It appeared to be a pure substance with a molecular weight of about 30,000 Constant antidiuretic, pressor and oxytocic activities were associated with this protein One U S P unit of each activity was about 60 micrograms or about one thirtieth of the potency of the pure polypeptides isolated by du Vigneaud and his co workers It is not known what proportion of the total oxytocin and vasopressin in the posterior lobe of oxen is represented by this protein The isolation of the pure substance was necessarily waste ful and only about 5 per cent of the original activity could be recovered Our present view is that a protein containing the polypeptides is stored in the posterior lobe of oxen Antidiuretic hormone in the urine of dogs underwent sedimentation in the ultracentri fuge as if part of a large molecule, however adsorption of the free hormone could not be excluded (Ames Moore & van Dyke 1950) The polypeptides are probably readily liberated from this protein by mild procedures What proportions of the total active polypeptides are free or protein linked is unknown although Rosenfeld s experiments suggest that the proportion of free oxytocin or vasopressin is low The question of liberation of the hormones in response to physiological demands is another aspect of the problem

How is the distribution of the hormones in the hypothalamus and posterior lobe modified by depletion of one or both hormones? Hild & Zetler (1953) and Zetler (1953) concluded that prolonged severe dehydration in the dog led to a marked depletion of vasopressin and antidiuretic hormone in both the hypothalamic divisions of the neurohypophysis and the posterior lobe The reduction in the concentration of oxytocin was less striking Their major conclusions from these and other experi ments were (*a*) vasopressin antidiuretic hormone and oxytocin are different hor mones and (*b*) the rate of repletion in the hypothalamus is most rapid for oxytocin and slowest for antidiuretic hormone Van Dyke Adamsons & Engel (1955) criticized their methods of biological assay and disagreed with their conclusion that antidiuretic hormone differs from vasopressin It is our belief that antidiuretic hormone and vaso pressin are identical and that better confirmatory experiments will have to be per formed before conclusions can be reached concerning the rates of repletion of oxytocin and vasopressin

Dicker & Tyler (1953) reported that the ratio of vasopressin to oxytocin in the posterior lobe of several mammals rises during parturition and especially during lactation For example in the dog *V/O* was found to be about 5 4 during lactation (4 dogs) in comparison with 1 0 in 8 normal dogs We also have investigated the distribution and *V/O* ratio in the divisions of the neurohypophysis in lactating dogs Results with 7 dogs were reported in 1955 recently the series has been expanded to 11 lactating dogs which as a group can be compared with a group of 12 normal dogs Only in the posterior lobe was there a significant fall in oxytocin (10 500 mU normal to 4 300 mU lactating P = 0 0002 and a rise in the *V/O* ratio (1 65 normal to 3 52 lactating P = 0 002) Antidiuretic hormone also was lower in the posterior lobe of lactating dogs (17 100 mU normal and 12 700 mU lactating P = 0 05) The amounts of hormones in divisions of the hypothalamus *except* the

supraoptic nuclei fell in lactating dogs but the variability of the results prevented the reaching of satisfactory conclusions (P = 0 14–0 18 for oxytocin and 0 10–0 13 for antidiuretic hormone) The ratio, *V/O* was not altered in any division of the hypothalamus The following conclusions are tentatively offered (*a*) oxytocin and antidiuretic hormone are depleted from the posterior lobe of the lactating dog, (*b*) the depletion of oxytocin is the greater and (*c*) assays in sufficiently enlarged groups of normal and lactating dogs *might* demonstrate a depletion of antidiuretic hormone in the paraventricular nuclei and tuber cinereum but not in the supraoptic nuclei Dicker & Tyler (1953) reported figures suggesting decreased storage of vasopressin in the posterior lobe of lactating rats, guinea pigs and cats but not dogs

Apart from experimental diabetes insipidus and extirpation of parts or the whole pituitary body, all other reports of depletion of the hormones experimentally are restricted to the posterior lobe It appears that when severe depletion of one hormone is produced such as a fall in antidiuretic hormone with dehydration the amount of the other hormone oxytocin, also is greatly reduced

Are both hormones liberated endogenously although the stimulus evoking their release appears to require only one? In a strict sense the answer appears to be affirmative and the evidence has been reviewed recently by Harris (1955) However the liberated hormones present in extremely minute amounts are usually detected by their effects on target organs such as the kidney the uterus or the lactating mammary gland Exogenous vasopressin or oxytocin have been injected to mimic the effects of endogenous hormones The estimates from such work suggest that although both hormones are liberated the proportion of oxytocin released is very much higher despite the fact that teleologically vasopressin may be the hormone the organism requires under particular experimental conditions Quantitative estimates of total amounts of released hormone or of the rate of release appear to be of little value if reliance be placed on assays of the hormones in the urine No satisfactory experiments in which vasopressin and oxytocin have been simultaneously measured in blood plasma or serum have been reported as a basis for deciding what amounts of the two hormones are liberated simultaneously We have failed in numerous attempts to secure information from the blood plasma of the external or internal jugular veins of anesthetized dogs receiving intracarotid injections of large doses of drugs such as nicotine salicylate or morphine sulphate Simple calculations reveal that relatively large quantities of hormones would have to be liberated if they are to be accurately determined in the jugular blood, even when this is restricted as far as possible to drainage of the brain Blood of the cavernous sinus may contain a very high concentration of hormones (e g 16 mU of antidiuretic hormone and 4 mU of oxytocin per ml of plasma) but a local injury of the posterior lobe or related structures has not been excluded

New information is available concerning the mammalian distribution of the free neurohypophysial hormones which du Vigneaud and his colleagues isolated The amino acid sequences of oxytocin and the two vasopressins are shown in Fig 2 In comparing the effects of lysine and arginine vasopressin van Dyke, Engel & Adamsons (1956) discovered that purified natural lysine vasopressin furnished by Professor du Vigneaud, differs from arginine vasopressin in only one important respect it has a much lower relative potency as an *intravenous* antidiuretic agent than arginine

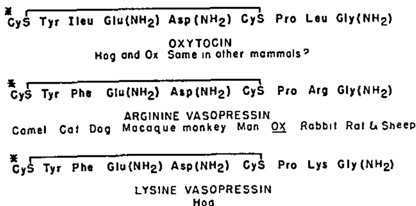


Figure 2

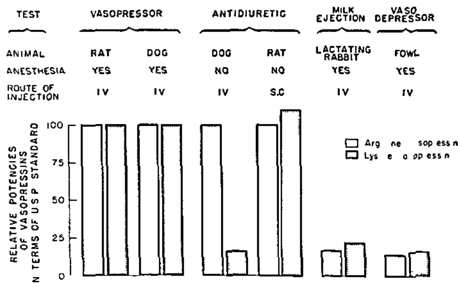


Figure 3

The relative potencies of arginine and lysine vasopressins The vasopressor potency of each hormone in the rat or dog in terms of U S P standard is given a value of 100 against which the other potencies are compared Extracts of hog posterior lobe powder have the same relative potencies as highly purified lysine vasopressin

vasopressin The relative potencies of the two vasopressins are illustrated in Fig 3 The highly purified vasopressins were obtained from the posterior lobes of cattle (arginine vasopressin) and hogs (lysine vasopressin) and there has been no similar product isolated from another species By determining the relative potency of a

posterior lobe extract as a vasopressor agent and as an intravenous antidiuretic agent good pharmacological evidence can be presented that the vasopressin present behaves like arginine vasopressin or like lysine vasopressin Such tests have been performed in extracts of the posterior lobe of the camel, cat, dog, hog, macaque monkey ox, rabbit, rat and sheep and of man Only hog extracts had a relatively low antidiuretic potency when injected intravenously as if they contained lysine vasopressin, the extracts of all the other mammalian posterior lobes caused elevation of the blood pressure and antidiuresis after intravenous injection as if they contained arginine vasopressin Proof that the vasopressin of the mammals other than the hog is arginine vasopressin will have to await the isolation of the hormone from each species and the elucidation of its composition and structure as has been done for the arginine vasopressin of the ox

SUMMARY

The distribution of neurohypophysial hormones in the hypothalamus and posterior lobe of the pituitary has been discussed Such information can describe how the hormones are stored but does not indicate at what rate they are secreted or liberated The dog among the mammals studied up to this time stores exceptionally large amounts of vasopressin (antidiuretic hormone) in the hypothalamus

The stored hormones of the posterior lobe of the ox appear at least in part to be linked to a protein with a molecular weight of about 30,000 To what extent this is true of other mammals is unknown The depletion of hormones stored in the posterior lobe, if severe probably affects both hormones although the teleological requirement may be for only one hormone Target organ responses suggest that both hormones are always liberated but that the proportion of oxytocin is much higher No successful simultaneous and quantitative determinations of both hormones in the blood have been reported

One oxytocin and two vasopressins, one containing arginine, the other lysine are known Only the hog, among mammals investigated appears to secrete lysine vasopressin

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Discussion

Chairman R Gaunt

Dale Did I understand Professor van Dyke to say that the ratio of vasopressin/oxytocin for the ox gland was 1 5 1? This is difficult to understand, because by definition one unit of vasopressin corresponds to the activity of 0 5 mg of the standard dry preparation of ox pituitary posterior lobe, and the same amount corresponds to 1 unit of oxytocin, so that the ratio for the ox pituitary *must* be 1 1, and nothing else, unless it can be suggested that the International Standard Preparation does not contain and yield to extraction oxytocin and vasopressin in the normal proportion

van Dyke Our conclusion depended upon the availability of pure or nearly pure oxytocin and vasopressin Having these available we could then decide to what degree vasopressin has oxytocic activity and vice versa Taking these facts into account we arrived at a ratio of 1 5 1

Dale It really involves re definition of what is meant by a Unit?

van Dyke That is correct

Dale That is a matter which quite frankly seems to me of rather serious importance

Heller Has Professor van Dyke compared the effects of intravenous injections of lysine and arginine vasopressin into rats as well as intravenous injections into dogs and subcutaneous injections into rats?

van Dyke No In our laboratory only Dr Ames has had conspicuous success with intravenous rat assays We find them too inconsistent and too time consuming

Ginsburg It would be interesting to see the effect of these vasopressins in the pig

Dale Could Professor van Dyke tell us whether he studied the residual oxytocic activity of his two vasopressins?

van Dyke Both preparations have some activity when tested for milk ejection effect, and for vasodepressor activity in the fowl There was no significant difference between the two preparations They were not tested on the uterus

Fromageot Professor van Dyke said that the two vasopressins act differently Lysine vasopressin depressed urine excretion very rapidly as compared with arginine vasopressin when tested on the same animals Could this be an after effect due to the arginine vasopressin being given first?

van Dyke Usually there is a very abrupt fall in the first five minutes to maximum even if there have been preceding injections of either lysine or arginine vasopressin The order of injections makes no difference

O Connor Professor van Dyke has mentioned the probable nature of the material released from the neurohypophysis into the blood stream, and referred to the findings of several different workers that it has a vasopressin oxytocin ratio of 0 01 to 0 25 This is a possibility of great interest to those concerned with renal function as it would indicate that enough oxytocic activity may occur in the circulation to make the effect of oxytocin on the kidney of importance particularly in relation to the excretion of sodium It occurs to me that in all of the examples where this vasopressin oxytocin ratio is so very low the stimulus was one which produced the *rapid* release of large quantities of hormone for example in dog the release of say 5 mU in a minute, as opposed to the steady release of 0 1 to 0 3 mU per minute which appears to be involved in the physiological control of renal function Could Professor van Dyke tell us whether at these slow steady rates of release, antidiuretic activity *is set free into* the blood stream without contamination with something like a hundred times as much oxytocic activity

van Dyke The figures I quoted were from the literature and were not our own We ourselves have found antidiuretic activity, together with oxytocic activity, in the blood drawn directly from the head chiefly from the external jugular with other vessels tied off The amounts produced are of little significance, because the doses of drugs used to cause release of hormones and injected into the carotid artery were unphysiologically high I cannot answer your question perhaps Dr Walker can help us

Walker It is a little difficult to comment on your remark out of the context of my paper, but I think that the relatively high values which Dr Bisset and I have been getting in the jugular venous blood of rats may be partly due to the fact that we have

taken rather large amounts of blood from both external jugulars and removal of blood has been shown by Ginsburg & Heller (*J Endocr* 9, 274, 1953) to be a potent stimulus for the release of antidiuretic hormone

Ginsburg To return to Dr O Connor s point, there is another difficulty involved in the conception that oxytocin is always secreted in excess of vasopressin Since the ratio of the hormones in the gland in undisturbed animals may be presumed to be constant, then the resynthesis of oxytocin would have to be many times faster than that of vasopressin, if it is released in larger amounts This corollary to the proposition that small physiological stimuli also liberates excess of oxytocin is not easily accepted

O Connor The amount of the hormone found in assays of the neurohypophysis is very large representing several days supply of hormone at the slow rates of release apparently involved in controlling renal function This release may be only a small fraction of the total turnover in the gland and so whatever its vasopressin-oxytocin ratio, would have little effect in determining the nature of the material needed to maintain the hormone content in the gland

Ginsburg I do not agree with Dr O Connor There must be a balance between the substances released and their repletion

G W Harris I wonder if Professor van Dyke would comment on the possibility that the different results we have heard regarding the oxytocin vasopressin content of the neurohypophysis in lactation, may be related to the suckling habits of the different species Is it possible that the oxytocin vasopressin relationships are not reduced in the cow or goat that are milked only once or twice a day but are reduced in the rat and dog in which the litter is left with the lactating mother throughout the whole day and night? If the stimulus to release of oxytocin is as seems likely a sensory stimulus to the nipple by the suckling young and perhaps also various conditioning reflexes set up by the presence of the young then it might be feasible that more oxytocin is released if the litter is present with the maternal animal both day and night as compared with the release if milking is performed on only two occasions through the twenty four hours

van Dyke I have no useful information to offer in reply to Professor Harris It appears that the rather excessive depletion of hormones in our dogs represented the demand imposed by nursing Our dogs and their litters were not carefully observed throughout the 24 hours of each day

G W Harris Were they with their litters continuously?

van Dyke Yes

Rossi Unpublished experiments of Malandra (Istituto di Anatomia e Istologia Patologica University of Pavia) have shown that, in water loaded lactating rats urine flow decreases significantly during the first hour after suckling and reaches normal levels after 3 hr In lactating but non suckling rats with the same experimental treatment there was no significant modification in urine excretion It seems

Heller Has Professor van Dyke compared the effects of intravenous injections of lysine and arginine vasopressin into rats as well as intravenous injections into dogs and subcutaneous injections into rats?

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The metabolism and fate of the neurohypophysial principles

by

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In a paper published in 1909, H H Dale showed that the intravenous injection of posterior pituitary extracts into cats resulted in the excretion of a pressor substance and concluded that the active principle is excreted in the urine' Twenty six years later Larson (1935) in a short note in the *Journal of Pharmacology* reported that the oxytocic as well as the pressor activity of posterior pituitary extracts was eliminated by the kidneys of anaesthetized cats and dogs and Heller & Urban (1935) in the same year showed that the urine of conscious rats into which posterior pituitary extract had been injected intravenously acquired a pronounced antidiuretic action Larson had found that amounts varying from 12 8 to 37 7 per cent of the initial dose were excreted and Jones & Schlapp (1936) in similar experiments on cats concluded that on the average 28 per cent of the pressor activity appeared in the urine

It was clear from these and later investigations (Heller, 1937 Ingram, Ladd & Benbow 1939 Larson 1939) that—irrespective of the species or dose used—only a part of the injected activity is eliminated What is the fate of the unexcreted portion? Is it destroyed and if so in which organ or organs of the mammalian body? The solution of this problem which does obviously imply the quantitative estimations of the neurohypophysial principles, i e of peptide hormones in body fluids and tissues meets formidable technical difficulties Chemical estimation of the active octapeptides is—in spite of the great advances made by du Vigneaud, Fromageot and their co workers—as yet hardly a practical possibility The use of labelled hormones has many limitations (discussed by Sonenberg & Money (1955) in connexion with the anterior pituitary hormones) It remains therefore to measure distribution and inactivation of the posterior pituitary principles by biological assay But it cannot be determined by biological assay of impure preparations whether one is dealing with small amounts of a very active substance or larger amounts of a less active derivative

THE STATE OF ENDOGENOUS AND EXOGENOUS POSTERIOR PITUITARY PRINCIPLES IN BLOOD AND URINE

The form or forms in which the active polypeptides are secreted into the blood is unknown Nor do we know in what form the native hormones circulate There are several possibilities Gomori positive substance has been seen in the blood vessels of the posterior lobe of the giraffe (Hanstrom 1952) the rat (Rothballer 1953) and the dog (Scharrer & Scharrer 1954) Do the hormones reach the blood linked with a

indicated therefore, when testing the neurohypophysial activity under these conditions, to consider carefully not only the duration of lactation but also the chronological relationship between the stimulus of suckling and the test

Folley Suckling under natural conditions by rats is not continuous but is interrupted by long periods of sleep, although of course, the suckling stimulus must be applied more frequently than in a goat I would suggest that Professor Fromageot runs an experiment with two groups of rats one with eight young and the other with say, only two young In this way, the influence of varying degrees of suckling intensity could be studied

Dale Alternatively, the kids could be left with the goats all day

Folley In our herd we do not usually allow the kids to suckle after the first few days, but of course it could be done if necessary

Dale I think it pertinent to ask how these experimental goats are killed Presumably they are not decapitated as is the case with the rat so that one cannot, perhaps, be sure what is happening to the blood supply of the head just at the time of killing

Folley In Mrs Dodd s experiments the goats were killed with a humane killer, taking care to avoid damage to the pituitary

Dale That does not of course stop the supply of blood to the head immediately

Folley My colleagues who killed the goats will be able to answer that

Fitzpatrick In this connexion could we ask Professor van Dyke how his camel was killed Could the procedure have influenced the results for the hypothalamus?

van Dyke Possibly The animal was killed by very rapid exsanguination the great vessels near the heart were quickly severed

Mrs Dodd I think it is important to consider the effect of the two forms of stimulation, namely demand suckling by the pups and milking on the vasopressin oxytocin ratios We wanted to know the influence of milking on the amount of oxytocin stored in the gland As far as the goats were concerned, as soon as a humane killer was applied to the head the carotid arteries were severed The glands were dissected out extremely rapidly and were in acetone in 90 sec to 4 min from death

van Dyke In the dog we insert a needle into the heart connect an indifferent electrode with the skin of the leg and kill by inducing ventricular fibrillation with electrical stimuli

instance it has been shown (Jones & Schlapp, 1936, Page, 1946, Croxatto *et al*, 1952) that lacked blood corpuscles inactivate the posterior principles, haemolysis may therefore influence the results So may dilution of the medium considering for example the recent finding of Miles & Wilhelm (1955) that simple dilution with isotonic sodium chloride solution causes the activation of a proteolytic system in guinea pig serum Nevertheless one important fact seems to emerge from all these investigations *In vitro*, both the pressor antidiuretic and the oxytocic activities of posterior pituitary preparations disappear much more rapidly from serum (Heller & Urban, 1935 Werle & Kalvelage, 1941, Birnie Eversole, Boss, Osborn & Gaunt 1950 Dicker & Ginsburg, 1950) than from plasma (Jones & Schlapp, 1936, Dicker & Ginsburg,

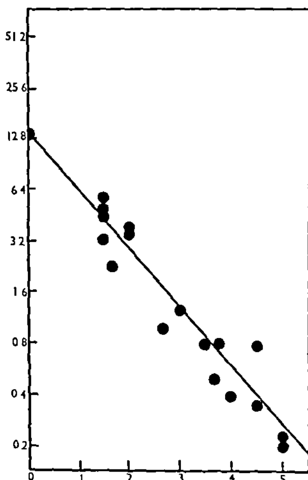


Figure 1

Concentration of vasopressin in arterial blood following the intravenous injection of 100 mU Pitressin/100 g into anaesthetized rats Ordinate concentration of vasopressin on a logarithmic scale (mU /ml) Abscissa time after injection in minutes (Ginsburg & Heller 1953*b*)

Gomori stainable carrier substance? Or are they liberated into the circulation as the large molecular compound which has been investigated by van Dyke, Chow, Greep & Rothen (1942) and Acher & Fromageot (1955)? Or is the inert protein to which —as Professor Fromageot told us this morning—the active peptides may be bound in the gland part of the secretory material? There is the further possibility that the peptides as such are released into the pituitary venous outflow But the release of the free polypeptides would not necessarily mean that they also circulate as small molecular compounds Arrival in the blood may only involve a change of carrier—the peptides attaching themselves to one or the other of the plasma proteins The work of Bennhold, Ott & Wiech (1950), Jancso (1955), Goldstein (1949) and others has by now familiarized us with the carrier function of plasma protein and at least one other hormone, namely thyroxine has recently been shown to circulate bound to a plasma globulin (Albright, Larson & Deiss 1955)

Binding of vasopressin and oxytocin by plasma protein is suggested by the finding (de Wesselow & Griffith 1934 Broun & Scheiner, 1935, Levitt, 1936, Heller, 1937, Croxatto Andrade & Barnafi, 1952) that the ultrafiltrability of the neurohypophysial principles is decreased when posterior pituitary extracts are mixed with blood It would also appear (Ginsburg & Heller, 1953*a*) that in the living animal the diffusion of vasopressin into the extravascular fluid phase (other than into that of the kidneys or organs of the splanchnic vascular area) is very slow This would not be expected of a compound of the molecular weight of about 1100 Moreover, Ames, Moore & van Dyke (1950) have found that the antidiuretic principle in the urine of dogs whose posterior pituitary had been stimulated by dehydration or the intracarotid injection of hypertonic sodium chloride solution undergoes sedimentation in the ultracentrifuge and that it can only partially be removed by pressure dialysis Similar results were obtained with urine of kangaroo rats (Ames & van Dyke 1950) Ham & Landis (1942) may have also been dealing with this large molecular compound or aggregate (see also Harris 1948) and Aujard Csanyi & le Breton (1955) have recently reported *on the presence of a non dialysable oxytocic substance in the urine of* a variety of mammalian species These findings suggest that some of the neurohypophysial activity released may reach the urine as a large molecular compound This compound may be the same as that isolated by van Dyke and by Fromageot from the posterior pituitary lobe but it has not been excluded that the small amounts of protein which normally escape into the urine (Rather, 1952) are sufficient to act as carrier for the neurohypophysial peptides

Sufficient has been said to indicate that we are not certain whether injected vasopressin and oxytocin circulate in the same form or state as the endogenous hormones And this in turn implies that results obtained with purified hormone preparations are not necessarily of physiological significance

THE INACTIVATION OF THE POSTERIOR PITUITARY PRINCIPLES BY BLOOD

The inactivation of neurohypophysial extracts by blood *in vitro* has been investigated by numerous investigators The results are divergent and even contradictory, mainly perhaps because the difference between using whole blood or serum or plasma has not been realized There are other factors which may have to be watched for

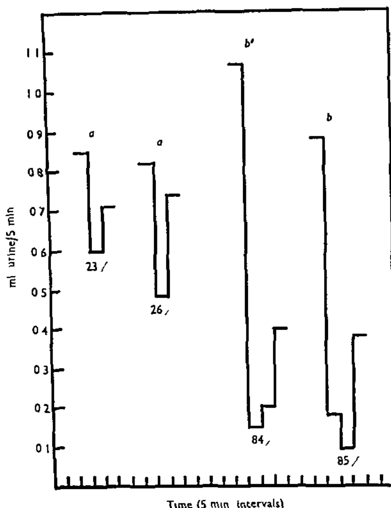


Figure 2

Incubation of rat blood (arterial) containing 10 units heparin and 1 mU Pitressin per ml for 30 min at 38 C Four point antidiuretic assay of mixture by the method of Ginsburg & Heller (1953c) Unanaesthetized rat with jugular and bladder cannulae a = i v injection of *non incubated* mixture containing 100 μU Pitressin a' = same volume of *incubated* mixture b = i v injection of *non incubated* mixture containing 300 μU b' = same volume of *incubated* mixture The figures above the abscissa indicate percentage antidiuresis About 100 per cent of the initial antidiuretic activity were recovered (Heller & Zaid unpubl)

It may be worth stressing that results obtained with tissues *in vitro* may only have a very limited application to the fate of a substance in the intact animal The reasons for this are not always sufficiently realized Firstly mechanical injury to cells by cutting mincing or grinding is likely to release or activate enzymes The rapid increase of amino acid and peptide nitrogen recently demonstrated by Conway Geoghegan & McCormack (1955) in ground rat kidney tissue may be mentioned as

1950, Sawyer 1954) The endogenous activities behave similarly, i e they also are more stable in plasma than in serum (Birnie *et al*, 1950, Ginsburg & Heller, 1953*a*)

It may be doubted whether these *in vitro* experiments with blood and its derivatives are of much physiological significance Even if one disregards the fact that in many of them enzyme systems may have been at work which are not active in circulating blood, inactivation *in vitro* is too slow to account for the disappearance of a substantial fraction of neurohypophysial activities from the circulation Figure 1 shows the rate of loss of injected antidiuretic activity from the circulation in the rat and Fig 2 shows the results of incubating rat blood (containing 10 units of heparin per ml) with vasopressin (Pitressin) Similar results had been obtained by Heller & Urban (1935) in rabbits and in cats by Jones & Schlapp (1936) who measured both pressor and oxytocic activity All these results were obtained in normal animals It cannot be excluded that there are conditions in which the rate of inactivation in circulating blood is so high that a significant portion of injected or released posterior pituitary hormones is inactivated Page (1946) has reported that an oxytocin inactivating enzyme occurs in the plasma of women near term in such high concentration that half of any quantity of posterior pituitary hormone injected intravenously would be inactivated in 45 sec His findings require confirmation

INACTIVATION BY TISSUES AND TISSUE EXTRACTS IN VITRO

Since even the earliest investigators could show that the posterior pituitary activities were not or only very slowly inactivated by blood it was reasonable to suspect that the hormones could be destroyed in the tissues Experiments with suspensions and homogenates of organs of various laboratory species showed some loss of antidiuretic pressor or oxytocic activity in all instances (Heller & Urban, 1935 Larson, 1938 Christlieb, 1940, Birnie, 1953, Sawyer 1954) but homogenates of small intestine, liver and kidney seem to be more potent than those of skeletal muscle brain and non pregnant uterus Cell free extracts of liver, kidney and spleen have also been shown to be effective (Heller & Urban 1935, Jones & Schlapp 1936 Eversole, Birnie & Gaunt 1949 Miller & Townsend, 1954) Heating or boiling the tissue or extract decreased or stopped inactivation Birnie (1953) has characterized the vasopressin inactivating system in liver homogenates to some extent, he found that its optimum activity was between pH 6 2 and 7 5 that the rate of inactivation increased appreciably with a rise in temperature from 0 to 37° C but that it sharply declined when the temperature was raised further The vasopressin inactivating principle was almost completely removed by adjustment to pH 5 2 or by half saturation with ammonium sulphate at pH 6 5 Addition of copper or zinc sulphate inhibited inactivation Birnie made no claims as to the specificity of his inactivating principle and further work would be needed to establish it The use of such terms as vasopressinase or pitocinase seems therefore premature All the more so since *in vitro* the posterior pituitary principles can apparently be inactivated by several mammalian enzymes Thus Croxatto and his co workers (Croxatto Croxatto Illanes & Salvestrini, 1942–43) have shown that both vasopressin and oxytocin are inactivated by chymotrypsin and in the presence of cysteine by spleen aminopeptidase and kidney hypertensinase Oxytocin is also destroyed by tyrosinase (de la Maza & Croxatto 1944) and vasopressin by trypsin (Croxatto *et al*, 1942–43 Lawler & du Vigneaud, 1953)

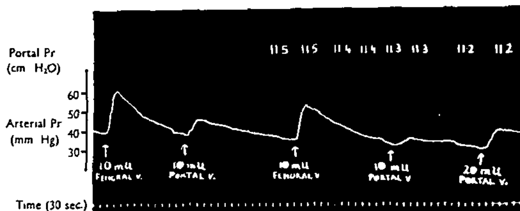


Figure 3

Comparison between the pressor effect of Pitressin injected into a femoral and into the portal vein Rat 250 g urethane dibenyline Injection needle inserted (not tied) into the portal vein. For further details, see text p 82 (Ginsburg unpubl)

accounted for at least 40 per cent of the vasopressin cleared These experiments did not make it clear which of the organ or organs of the splanchnic bed remove the hormone But it would appear (Table 1) that the intestine removes none or very *little of the endogenous antidiuretic principle and similar results (Ginsburg 1956)*

TABLE I

Antidiuretic activity of blood from the gastro intestinal outflow
(Ginsburg unpublished)

Experiment no	Antidiuretic activity (mU /ml) in blood from	
	Abdominal aorta	Portal vein
1	0 30	0 32
2	0 19	0 14
3	0 37	0 52
4	$<$ 0 04	$<$ 0 04

All animal in pentobarbitone anaesthesia Rat 4 was neurohypophysectomized. Vena cava t ed below renal veins Blood pressure maintained by compensation at 100 mm Hg

have been obtained with injected vasopressin These results suggest strongly that in the rat the liver participates in the removal of vasopressin However it is not the only or perhaps not the main organ which clears the neurohypophysial principles from the circulation Experiments on nephrectomized rats (Ginsburg & Heller 1953*b*) showed that the kidneys extracted about 50 per cent of the injected antidiuretic activity Crawford & Pinkham (1954) using a wide range of doses and Dicker (1954) confirmed these results

an example Secondly, access and therefore perhaps velocity and/or completeness of inactivation of an added substance will, in the absence of transport by circulating blood or lymph, to some extent be dependent on the degree of dispersion of the isolated tissue, i e permeation of a test substance will differ in homogenates and in slices of the same tissue Reaction velocity is probably also influenced by the release of permeability increasing polypeptides liberated from tissues by short term action of proteolytic enzymes (see Spector, 1951) Lastly the demonstration that an organ or tissue inactivates a substance *in vitro* can hardly be applied to the intact animal unless it has been shown that the substance whose fate one studies has access to that organ or tissue *in vivo* That is to say, the organ or tissue 'clearance' for the substance has to be determined for example there seems little sense in incubating a test substance with brain homogenates unless it can be shown that it penetrates the brain blood barrier

FATE OF THE NEUROHYPOPHYSIAL PRINCIPLES IN ORGANS IN SITU AND IN THE INTACT ANIMAL

Only one group of workers (Eser & Tuzunkam, 1950) seem to have estimated neuro hypophysial activity after perfusion through an isolated organ They reported that the antidiuretic activity of posterior pituitary extract in tyrode was reduced by 70 per cent when circulated through guinea pig liver for 3 hr , less clear cut results were obtained with kidneys However most workers interested in the fate of the posterior pituitary principles *in vivo* choose other techniques Eversole, Birnie & Gaunt (1949) found that 40 mU Pitressin injected into the spleen of hydrated rats inhibited water diuresis to a lesser degree than the same dose injected subcutane ously or intramuscularly smaller doses failed to give clear results Similarly, Møller Christensen (1951) comparing the effects of intrajugular and intrasplenic injections of posterior pituitary extracts on the blood pressure of anaesthetized rabbits and cats found that the latter were much less effective These experiments are open to the objection that an injection into a tissue (even a parenchymatous one like the spleen) may not be comparable to an injection into the blood It is therefore interest ing that Mathe & Altman reported recently (1954) that small doses of vasopressin injected into the splenic vein of unanaesthetized dogs in water diuresis had the same effect on the urine volume and urine concentration as an injection into the saphenous vein Figure 3 on the other hand shows an experiment of my colleague Dr Ginsburg in which Pitressin had been injected directly into the portal vein The doses used, although not influencing portal pressure significantly raised the arterial pressure much less than the same doses injected into the femoral vein It is difficult to believe that the dog and the rat handle vasopressin in so different a manner More work is needed in both species

In the rat evidence on the fate of exogenous and endogenous vasopressin obtained by a different approach is already available Measuring vasopressin concentration in the blood by assays for antidiuretic activity, Ginsburg & Heller (1953*b*) could show in anaesthetized rats that tying of the coeliac and mesenteric arteries (which was demonstrated to cut the blood flow through the liver by about 70 per cent) retarded the removal of injected vasopressin from the circulation significantly With the doses and under the experimental conditions used the splanchnic vascular area

irreversibly inactivated or a combination of these processes may apply It has been mentioned that the injection of vasopressin results in the excretion of an antidiuretic (and pressor) substance in the urine but experiments in rats (Ginsburg & Heller, 1953*b*) indicate that only a fraction of the activity cleared by the kidneys appears in the urine Dr S M A Zaidi and I in some recent experiments have attempted to find an explanation for this descrepancy We first extracted the kidneys of normal rats i e adult male animals which were not in water diuresis and which had been killed by decapitation either with acid saline or by a method employing acid ethanol similar to that used by Vogt (1953) for the hypothalamus and Bisset & Walker (1954) for blood It has the advantage that—when tested in rats—the depressor effect commonly obtained with aqueous extracts is no longer seen The antidiuretic effect of a given dose of vasopressin remains unchanged when added to such kidney extracts (Fig 4) thus excluding the presence of antagonistic or potentiating substances The mean recovery of vasopressin added to rat kidney homogenate (as estimated by 4 point assays for antidiuretic activity using a modification of the method of Ginsburg & Heller (1953*c*) was 79 7 ±3 49 per cent with aqueous extracts and 73 9 ±3 40 per cent with alcoholic extracts The mean recovery of small amounts (5 mU/kidney) of vasopressin injected into a kidney immediately after death was 90 9 ±8 23 per cent However as shown in Table II no antidiuretic effect could be obtained with the

TABLE II

Antidiuretic assays of acid ethanol extracts of kidneys of normal rats

No of extract	Sensitivity of assay rat to Pitressin in μU /animal	Volume injected* without antidiuretic effect (in ml)	Maximum antidiuretic activity in both kidneys (in terms of mU Pitressin)
1	6 25	0 8	< 0 23
2	6 25	0 8	< 0 23
3	6 25	0 4	< 0 46
4	6 25	0 3	< 0 62
5	6 25	0 3	< 0 62
6	6 25	0 3	< 0 62
7	6 25	0 4	< 0 46
8	6 25	0 4	< 0 46
9	3 13	0 4	< 0 23

Total olume of each extract = 30 ml

extracts of normal rat kidney That is to say when expressed in terms of the limits of sensitivity of the assay the kidneys contained much less than the equivalent of 220μU (corrected for incomplete recovery) vasopressin per gramme kidney tissue This does not suggest that the antidiuretic hormone is stored for long after it has been cleared by the kidney Nor could any accumulation of activity be demonstrated in the kidneys of rats whose blood concentration of endogenous vasopressin had been artificially raised by ether anaesthesia (Fig 5)

When both kidneys had been removed and the coeliac and mesenteric arteries had been tied, the clearance of injected vasopressin was only about 10 per cent of that of intact rats (Ginsburg & Heller, 1953*b*) suggesting that organs other than the kidneys and those in the splanchnic circulation did not participate to any great extent in the removal of vasopressin This agrees with results in animals whose blood level of endogenous vasopressin had been raised by ether anaesthesia the antidiuretic potency of plasma obtained from the inferior vena cava below the renal and hepatic venous inflow was less than one third of that of arterial plasma (Ginsburg & Heller, 1953*a*) which indicates that for the endogenous hormones also the kidneys and organs of the splanchnic vascular bed are major sites of clearance from the blood

Assuming then that in the rat the kidneys and the liver remove the bulk of circulating vasopressin, how do these organs handle the hormones which they have cleared? There are obviously several possibilities The hormone (either the polypeptide as such or a polypeptide carrier complex) could be temporarily stored or reversibly bound it could be excreted (into the urine or the bile), it could be

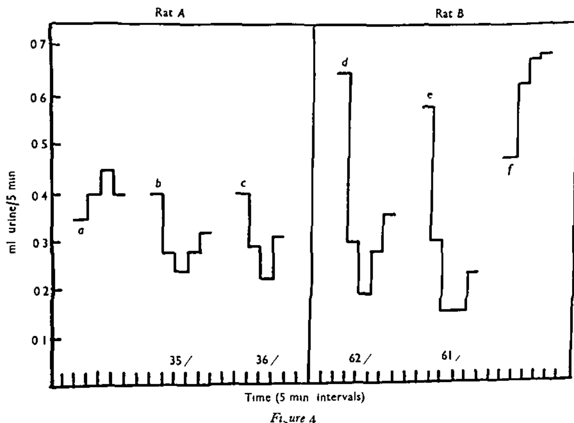


Figure 4

The antidiuretic effect of Pitressin added to acid ethanol rat kidney extract One ml of extract was equivalent to 75 mg wet kidney Rat *A* *a* = i v injection of 0 4 ml kidney extract *b* = 0 4 ml extract containing 6 25 µU Pitressin *c* = 6 25 µU Pitressin in 0 4 ml 0 9 per cent NaCl solution Rat *B* *d* = 100 µU Pitressin in 0 3 ml 0 9 per cent NaCl solution *e* = 100 µU Pitressin in 0 3 ml kidney extract *f* = 0 3 ml kidney extract The figures above the abscissa indicate percentage antidiuresis It will be seen that adding kidney extract did not alter the antidiuretic potency of Pitressin (Heller & Zaid unpubl)

To study the fate of vasopressin in the kidney further, 100 mU Pitressin per 100 g rat—the same dose as that used by Ginsburg & Heller (1953*b*) in their clearance experiments—was intravenously injected into unanaesthetized rats, the animals killed at given times after the injection and the antidiuretic activity in their kidneys estimated. Table III shows the recoveries expressed as percentage activity of the dose injected: some small amounts of an antidiuretic principle could sometimes be recovered from the kidneys of animals killed 1 to 3 min. after they had been injected, but only traces from those killed after a longer interval. It appears then that the bulk of the 100 mU. or so which the kidneys of our 200 g. rats are likely to have removed from the blood was either excreted or inactivated or both within the first few minutes after injection. It would seem that both mechanisms are involved and that both operate with great rapidity. The results shown on Table III referred to extracts of kidneys from which pelvic tissue and ureters had been carefully removed. But when, post mortem, the ureters were tied at their junction with the bladder and

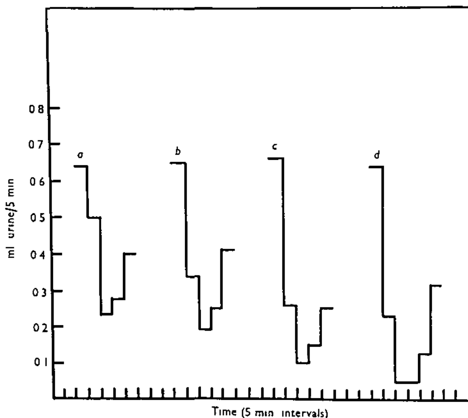


Figure 6

Antidiuretic assay of extract of kidneys (including pelves and ureters) of an unanaesthetized rat killed 3 min. after the injection of 100 mU. Pitressin/100 g. *a* = 50 μU. Pitressin i.v. *b* = 0.2 ml. kidney extract i.v. *c* = 100 μU. Pitressin. *d* = 0.4 ml. kidney extract i.v. All injections made up to same volume. The kidney extract contained antidiuretic activity equivalent to 4.1 per cent. of the dose of Pitressin injected into the donor rat. (Heller & Zaidi, unpubl.)

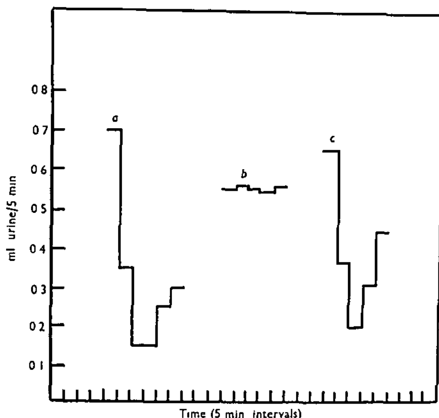


Figure 5

Absence of demonstrable antidiuretic activity in the extract of the kidneys of a rat anaesthetized with ether for 10 min and injected with Pitressin (100 mU/100 g body weight) during the 7th min of anaesthesia Recipient rat unanaesthetized jugular and bladder cannulae $a = 50\,\mu U$ Pitressin in 1 0 ml 0 9 per cent NaCl solution i v $b = 1$ 0 ml kidney extract $c = 25\,\mu U$ Pitressin in 1 0 ml 0 9 per cent NaCl solution Total volume of kidney extract = 20 ml Hence kidneys contained the equivalent of less than 0 5 mU Pitressin (Heller & Zaidi unpubl)

TABLE III

Unanaesthetized rats injected with 100 mU Pitressin/100 g
Antidiuretic activity of acid ethanol extracts of kidneys

Min killed after injection	Percentage recovery of injected dose			
1	< 0 12	< 0 12	< 0 24	< 0 24
3	0 20	0 26	< 0 12	< 0 12
6	< 0 45	< 0 20	< 0 45	
12	< 0 50	< 0 25	< 0 25	
15	< 0 50	0 09		

differential centrifugation of kidney suspensions according to Lowell, Greenspon, Krakower & Bain 1953) suggest that the glomeruli are not implicated (Fig 7)

Similar experiments as with kidney extracts were also done with extracts of rat liver The mean recovery of vasopressin added to liver suspension was 88 2 ±4 46 per cent Figure 8 shows that endogenous activity was also recoverable However no antidiuretic effects were obtained with extracts of the liver of normal animals indicating that the storage of antidiuretic hormone (if any) was less than about 70 μU /g liver Table IV shows the results of antidiuretic assays of liver extracts of unanaesthetized rats injected with 100 mU /Pitressin/100 g The animals were killed at intervals varying from 1 to 15 min after the intravenous injection Some small amounts of antidiuretic activity (equivalent to less than 1 per cent of the dose given) were found in the livers of rats killed up to 3 min after the injection but extracts of livers of

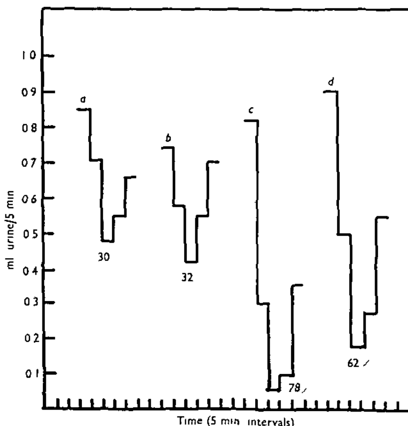


Figure 8

Recovery of endogenous antidiuretic hormone in rat plasma when added to rat liver suspension and extracted by the acid ethanol method The plasma concentration of antidiuretic hormone was raised by anaesthetizing the donor rat with ether Antidiuretic assay *a* = 0 ml jugular plasma of rat anaesthetized with ether i v *b* = 0 2 ml of extract of liver homogenate mixed with the jugular plasma (1 ml extract was equivalent to 00 mg liver plus 1 ml plasma) *c* = 0 4 ml plasma *d* = 0 4 ml extract Percentage recovery of antidiuretic activity = 88 4 (Heller & Zaidi unpubl)

extracts were made of kidneys including 'pelvis and ureter (i e when the dead space of the kidney was considered), quite substantial amounts of antidiuretic activity could be recovered Figure 6 shows the results of such an experiment in which the rat was killed 3 min after the injection of vasopressin Further experiments showed that in that time small amounts of antidiuretic activity (0 39 0 64 and 0 53 per cent of the injected dose in 3 experiments) had also reached the bladder The total recovery from renal tissue, ureters and bladder was equivalent to about 5 per cent of the injected dose at a time when 25 per cent or more of the injected dose had been removed by the kidneys The very great rapidity of inactivation of vasopressin in the rat kidney thus indicated (which would be faster than the rate of inactivation observed *in vitro*, Dicker & Greenbaum, 1954, Zaidi, 1955 Heller & Zaidi, 1956) is perhaps less startling if one considers that 200 mU of vasopressin (the order of total dose injected into our rats) represent only about 0 4 μg in terms of the pure octapeptide We cannot say in which tissue element the inactivating process occurs but experiments in which vasopressin was incubated with homogenates of the 'glomerular and the 'tubular' fraction of rat kidney (prepared by

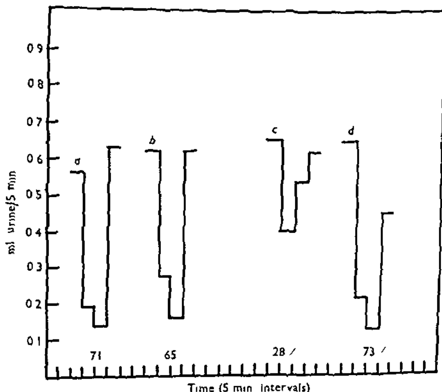


Figure 7

Effect of incubating Pitressin with the glomerular and the tubular fractions of rat kidney (see text p 89) for 30 min at 38 C Antidiuretic assay *a* = i v injection of 200 μU Pitressin in glomerular fraction *after* incubation *b* = 200 μU Pitressin in glomerular fraction *before* incubation *c* = 220 μU Pitressin in tubular fraction *after* incubation *d* = 220 μU Pitressin in tubular fraction before incubation The figures *above* the abscissa indicate percentage antidiuresis The amount of Pitressin added to the fractions was proportional to the weight of suspended renal tissue (Heller & Zaidi unpubl)

TABLE V

Amount of antidiuretic activity excreted by a rat infused with urine collected from another rat injected with vasopressin

Rat A		
500 mU Pitressin i.v.	0·1 ml urine A*	0·544 mU
	Thus 3·3 ml urine infused into rat *B* had a total antidiuretic activity equivalent to	17·952 mU
Rat B		
3·3 ml urine of Rat *A* i.v.	0·1 ml urine *B*	0·056 mU
	Thus 3·55 ml urine excreted by rat *B* had an antidiuretic activity equivalent to	1·992 mU
Result Rat *B* excreted 11 per cent. of the infused activity		

The volume of urine collected from rat *A* during and for one hour after the infusion was 4·0 ml. The urine was then made isotonic with a 0·9 per cent. NaCl solution which involved dilution to 5·0 ml. One portion (i.e. 1·3 ml.) of the diluted urine was used to estimate antidiuretic activity by the method of Ginsburg & Heller (1953*c*). It was found that rat *A* had excreted the equivalent of 27·2 mU. vasopressin or 5·44 per cent. of the infused activity. The remaining portion (= 3·3 ml.) was infused into rat *B*.

In view of our findings it seems preferable for the present to assume that the injection or the release of vasopressin results in the rapid excretion of small amounts of the unchanged hormone. But be this as it may, the problem of the intermediary metabolism of the posterior pituitary principles remains and is a challenge for future work. Such work is—in our opinion—desirable not only because of its intrinsic interest but also because the increasing number and importance of biologically active polypeptides raises the question of a common metabolic path of these compounds. Only one of them, namely insulin, has been investigated to a similar extent as vasopressin. The results indicate that as in the case of vasopressin the kidneys and the liver are major sites of clearance and inactivation for intravenously injected insulin (Haugaard, Vaughan, Haugaard & Stadie, 1954; Elgee & Williams, 1954). The renal excretion of insulin, on the other hand, seems to be of much less importance than that of the posterior pituitary hormones.

There is a further reason for work on the metabolism and fate of the neurohypophysial hormones: some years ago several groups of workers suggested that the impaired water diuresis, the oedema and the ascites in patients with liver cirrhosis were due or partly due to decreased inactivation of the antidiuretic hormone by the diseased liver. They based their hypothesis on the demonstration that liver homogenates or extracts inactivate vasopressin. They did not consider that it had also been shown (Jones & Schlapp, 1936; Larson, 1938; Christlieb, 1940) that homogenates of other tissues do this as well. Moreover, there seems to have been no hesitation to apply *in vitro* results with animal tissues to intact human beings. It seems to us that if an increase in the blood level of a posterior pituitary principle (or perhaps of any hormone) is to be ascribed to decreased inactivation, the organs or tissues which remove and metabolize the endogenous active principle should be known and that

TABLE IV

Unanaesthetized rats injected with 100 mU Pitressin/100 g
Antidiuretic activity of acid ethanol extracts of liver

Min killed after injection	Percentage recovery of injected dose			
1	< 0·47	< 0·46		
3	0·61	0·46	< 0·43	< 0·12
6	< 1·0	< 0·43	< 0·48	
9	< 0·48	< 0·48		
12	< 0·50	< 0·50		
15	< 0·52			

animals killed *after 6 min and later had no antidiuretic effect If therefore*—as indicated by the experiments of Eversole, Birnie & Gaunt (1949), Ginsburg & Heller (1953*b*) and the recent work of Ginsburg (Fig 3)—the liver of our rats had cleared a substantial fraction of the injected hormone within that period it must be inferred that the retained vasopressin was rapidly inactivated

It would thus appear from these investigations that in the rat, the kidneys and perhaps the liver are not only the main organs of vasopressin clearance but also major sites of the metabolic degradation of the hormone Whether the kidney in addition to clearing and inactivating vasopressin also excretes the unchanged polypeptide cannot be answered as long as we do not know whether inactivation in the tissue does give rise to breakdown products of the hormone with lower biological activity The renal excretion of such a derivative has been postulated by Dicker & Greenbaum (1954)

The main basis of their claim is as follows they infused vasopressin into rats anaesthetized with ethanol and found that the antidiuretic activity of urine collected from such animals remained unchanged after incubation with rat kidney slices but that it was almost entirely destroyed when incubated with liver slices When the urine of a rat injected with vasopressin was infused into a second rat the antidiuretic activity of the urine of the recipient was only about half that present in the infused urine They suggest that the kidneys and the liver in the intact rat behave like slices of these organs *in vitro*, in other words that half of the antidiuretic activity in the urine was destroyed by the liver and that the other half passed unchanged through the kidneys Since rats infused with Pitressin excrete, according to Dicker (1954) only 3–28 per cent *of the injected dose the results of these experiments would be strongly* in support of their thesis They were therefore repeated Table v shows one of our experiments It will be seen that the recipient excreted 11 per cent of the activity in donor s urine, i e the animal excreted much the same amount as if it had been infused with Pitressin These results do not support Dicker s & Greenbaum's hypothesis

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the quantitative aspect of the problem should not be neglected Our results suggest that organs like kidney and liver *in situ* inactivate large doses of injected vasopressin with great rapidity Pathological changes in these organs would presumably have to be very pronounced before their capacity to deal with relatively small quantities of the endogenous principle is critically lowered It will also have to be seen to what degree a gradual decrease in the clearing and inactivating capacity of a diseased organ can be compensated in another site I submit that only if approached in this comprehensive manner, can studies in the fate and metabolism of the neurohypophysial hormones be expected to be a valid aid to clinical research

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I thought that the reason might be that a significant part of the vasopressor effect of the Pitressin was due to vasoconstriction in the kidneys, and although I had eliminated one of the organs by which the Pitressin is cleared, I had also removed one through which its pressor effect is exerted

Gross How long was this after you had taken out the kidneys?

Ginsburg Less than 30 min

Gross We did it the day before

Ginsburg It does not make it any clearer

Grose Which dose did you use?

Ginsburg About 10 or 15 mU

Fromageot There are several reactions by which the antidiuretic hormone may be inactivated It may be subject to proteolysis or deamination or to inactivation by the oxidation of SH groups or the tyrosine residues I would like to ask Professor Heller about the conditions of his inactivation experiments Were they carried out in the presence or absence of air? If air was present, was the supply of it adequate to permit oxidation?

Heller We have not investigated the chemistry of the inactivation process but we have considered some of the possibilities you have mentioned With regard to your second question, most of the *in vitro* experiments were done at 37 to 38 C under aerobic conditions but the tubes were neither shaken nor were their contents stirred with oxygen

Croxatto I would like to put a question regarding the vasopressinase and oxytocinase When you are referring to extracts of tissues I agree that you are not dealing with specific enzymes but it is a different thing when we are dealing with the blood of pregnant women In this case the inactivating potency may be due to a specific enzyme We have seen only small change in the hypertensinase activity although the vasopressinase and oxytocinase activities increase very much in the last month of pregnancy

Heller I think perhaps that Professor Croxatto has misunderstood me There can be no doubt about the physiological significance of the enzymes or enzyme systems mentioned and it may be convenient to talk of vasopressinase but I would rather reserve this name for an enzyme whose specificity has been clearly demonstrated As to calling an enzyme Pitocinase I don t believe that Nature has gone to the trouble to produce an enzyme with inactivates solely a preparation of Parke Davis and Co

Wirz I have a question which I might as well ask here as it doesn t fit in with any other topic to be discussed I have been asked for advice in Basle about a case of diabetes insipidus which does not respond to Pitressin One might think of an increased rate of destruction of the Parke Davis preparation but does Professor Heller or anyone else know what to do about such a patient? What is the explanation for this condition and what are the therapeutic possibilities? It is an infant of five months

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Discussion

Chairman J M Yoffey

de Wardener I am a little puzzled by the rapid disappearance of antidiuretic activity from the blood and the kidney parenchyma The antidiuretic activity on the functioning kidney goes on 20–30 min and even longer, yet the substance which is producing this change has disappeared from the blood and kidney in from six to seven minutes

Dale That applies to the pressor effect which also lasts for much longer than six or seven minutes

Heller I think there may be two explanations We have been investigating the fate of relatively large doses of vasopressin but the amount needed to produce a biological effect—as has already been said this morning—is very likely to be measured in micro units Such small amounts of the hormone may still have been present in the tissues but we would not have been able to demonstrate them Another explanation, of course for which however I have no evidence would be that the biological effect persists after the agent which elicited it has disappeared

Gross In connexion with other work on nephrectomized rats, we injected Pitressin and found an increase and prolongation of the pressor effect Another polypeptide—angiotonin—did not give this reaction We regard this as evidence in support of the view that Pitressin is inactivated by the kidneys

Ginsburg This is extremely interesting and odd because I have observed pressor responses to Pitressin in nephrectomized rats and did not get a prolongation of the effect

The excretion of posterior pituitary principles in the urine

by

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ONE of the less direct approaches for obtaining evidence of the participation of the neurohypophysis in physiological processes has been the study of the excretion of antidiuretic substance (ADS) in the urine From a consideration of the numerous conflicting reports in the literature of the multiplicity of clinical conditions which reputedly are associated with large amounts of antidiuretic hormone in the urine one forms the impression that either the posterior pituitary is associated with innumerable metabolic processes in the body or more reasonably that the methods used for extraction of the urine have been highly inadequate and non specific Some years ago various extraction methods were attempted in the laboratory in London of Sir Charles Dodds At this time it was found that such procedures as dialysis or precipitation yielded extracts which gave highly erratic and poor recoveries (Noble Rinderknecht & Williams 1938) These inconsistencies are now realized to be related to whether or not the active molecule will dialyse at a uniform rate through various membranes and if it can be quantitatively extracted off the membrane when it does not dialyse (Walker, 1939 Schaffer, Cadden & Stander 1941 Ames Moore & van Dyke 1950) As a result however of such unsatisfactory studies a new method of extraction of urine was explored and published in 1939 (Noble Rinderknecht & Williams)

This method employing the principle of adsorption of the active principle on zinc ferrocyanide was found to yield a good recovery of antidiuretic hormone after it was added to urine but the method was not used in serious attempts to study posterior lobe function

More recently a continuation of the study of extraction methods has been made by Dr Taylor and Dr Jessup and these have been applied in the study of neurohypophysial function Initially I would like to discuss the zinc ferrocyanide method of extraction and later include some preliminary observations on what we believe is a new improved method During the years which we have been using this first method of extraction we have realized that we were stumbling over obstacles of an ill definable nature hidden in a morass of impurities which could not be clarified until an appreciation of the properties of the isolated and identified active principles was accomplished These more primitive observations therefore left us exposed to the criticism of non specificity of the extraction method These shortcomings have been appreciated to the extent that these experiments have had to be stripped down and the results expressed in the barest and most conservative form They are familiarly

which has to be maintained on a constant infusion If the infusion stops for some reason the child is quite feverish the next morning

Heller These cases are rare but a number of them have been described and they seem to be genetically determined Williams & Henry (*Ann int Med* 27, 84, 1947) have investigated the possibility of increased inactivation of vasopressin, but I don t think that their experiments were quite decisive Judging from the amount of hormone which can be inactivated in normal rats in a very short time, it seems unlikely that increased inactivation is the trouble It seems much more likely that the defect is in the kidney which cannot respond to vasopressin in the normal manner

Gaunt Has the steroid production been determined in this infant? One can get high Pitressin resistance from chronic administration of steroids

Lloyd We have studied a child like this I think first one must establish that a Pitressin fast individual does not respond to a large dose given intravenously We have seen a number of cases of diabetes insipidus in which the patient became refractory to intramuscular or intranasal administration but responded when it was given intravenously As Dr Gaunt says, we have studied one patient in particular who had a corticosteroid excretion which was three times higher than normal when he was requiring very large amounts of Pitressin A year later, for some reason he was requiring much less and corticosteroid secretion was then within the normal limits The major problem in the child with nephrogenic diabetes insipidus is that posterior pituitary substance is ineffective A decrease in solute intake will produce a modest decrease in urine volume Apart from this all you can do is to keep them on water day and night

Ginsburg I have often wondered whether the uptake of Pitressin by the kidney is related to its antidiuretic action It might be a good idea to test this in a case such as we are now discussing by finding out whether a higher proportion of the injected dose is excreted in the urine

Heller Dr Darmady tells me that he has dissected the tubules in a case of vasopressin resistant diabetes insipidus and that, at least, microscopically he could find no difference between the tubules of such a child and those of a normal child That of course does not necessarily mean that they were functionally normal

de Wardener One thing about these children is that if they do get very dehydrated they can apparently concentrate the urine to a specific gravity of 1,023

Wirz No, in our case it was not as high as that, about 1 016 but I don t know how far they went with the dehydration

Yoffey I wonder if anyone has ever tried coupling the polypeptides with a fluorescent molecule?

Heller No this has not been done with posterior pituitary hormones but it seems a very useful suggestion

antidiuretic hormone) In addition, intraperitoneal or subcutaneous injections of a concentrated extract devoid of antidiuretic hormone may be followed by an anti diuresis due to toxic substances, or a high salt content Because of such factors van Dyke (van Dyke Adamsons & Engel, 1955) has stressed that all assays of extracts or body fluids not rich in antidiuretic hormone should be assayed by an intravenous technique One property of urine extracts however, has suggested that they may not be suitable for intravenous assay of the antidiuretic hormone When such extracts of urine from any source are given intravenously they show a marked and sustained pressor action similar to that shown by vasopressin The degree of pressor activity when compared with antidiuretic activity, precludes the possibility that the action is due to the antidiuretic hormone and in addition the pressor substance is resistant to heat at pH = 10 (Noble Plunkett & Taylor, 1950) It is possible that this pressor contaminant may be piperidine (von Euler, 1945) or more likely the substance studied by Dekanski (1951) Since extracts of urine therefore exert a pressor effect when given intravenously it was feared that this would result in an antidiuresis from a non specific effect on renal circulation It is possible that this belief is ill founded and Dr Taylor and his collaborators are presently engaged in testing urine extracts by a modified Dicker (1953) technique

Assays to be reported in this paper therefore, have all been performed on the basis of the method of Burn (1937) and the rats injected by the subcutaneous or intra peritoneal route An adapted modification in the use of the fed rather than the starved rat is however of some interest (Jessup & Taylor 1954) Apparently the starved animal, even though having access to water becomes partially dehydrated and has endogenous liberation of antidiuretic hormone As a result the induced water diuresis starts more slowly and is more variable than that obtained by using fed animals and in addition the preparation is less sensitive to antidiuretic hormone An added feature of using fed animals is that they may be used every second or third day for assay purposes

Using the described extraction method and means of assay, what is the evidence which led us to believe that it would be possible to use the results of urine assays in an interpretation of posterior lobe function even though the active substance could not be positively identified as the active factor of the extracts? Again one may question whether because antidiuretic hormone added to urine may be recovered by a certain extraction method there is any assurance that the hormone excreted under physiological conditions is in the same form so that it also would be extracted? Certainly Heller (1937) has demonstrated that posterior lobe hormone may be bound or adsorbed in some fashion by body fluids, and something of a similar nature might occur in urine Information on these problems may be derived from two different types of experiments The first concerns the appearance of extractable ADS in the urine of humans immediately after the intravenous injection of the hormone and the second the gradual increase of antidiuretic activity in the urine of rats and human subjects with increasing dehydration The study of such conditions associated with an excretion of ADS in the urine is of course in no way an original contribution but is now an accepted conclusion drawn from published experiments in which many of you here today have collaborated

In the first experiments Dr Taylor was fortunate in being able to collaborate with

referred to in the laboratory as the *BVD*' or '*BDV* type of experiment—implying of course their crudity and non specificity, since they were 'Before van Dyke' and Before du Vigneaud

The zinc ferrocyanide method, which can only be profitably applied to urine, consists of the formation of a voluminous flocculent precipitate resulting from the addition and interaction of sodium ferrocyanide and zinc sulphate, which at an acid pH adsorbs posterior lobe hormones They can then be eluted from the separated precipitate by ammoniacal ethanol After concentration impurities may be pre cipitated by high concentrations of ethyl alcohol The final aqueous extract, when concentrated to 5 or 1 per cent of the original volume of urine (which is always adjusted to a constant specific gravity of 1,010) is a dark brown homogeneous mixture from which the only thing we have isolated is urea Surprisingly enough, however, the recovery by this method of antidiuretic hormone added to urine is satisfyingly constant and gratifyingly high (Noble & Taylor, 1953)

In an experiment to depict the effectiveness of this extraction process, from 1 to 10 U of the vasopressor principle may be added to 3 l of urine (Noble, Plunkett & Taylor 1950) For comparative results extraction is made of this urine sample as well as from a control aliquot of urine but without added antidiuretic hormone The hormone preparation is then added to the final extract of the control urine and the samples assayed Using the Burn method of assay and administering the extracts by either subcutaneous or intraperitoneal injection it is found that the difference be tween the activities of extracts to which antidiuretic hormone was added before and after extraction is about 15 per cent A recovery of over 80 per cent therefore, is consistently experienced

Such an experiment in addition demonstrates a curious feature which is consist ently shown with extracts of urine When tested by subcutaneous injection the total apparent recovery is found to be increased by a factor up to 5 This effect is appar ently related to augmenting substances in the extract which by prolonging absorp tion, result in an increased sensitivity of the method by some 5 times Fortunately this augmentation is constantly shown by urine extracts so that by using suitable control urine solutions with graded amounts of antidiuretic hormone added after extraction it is still possible to obtain quantitative data Intraperitoneal injections are not followed by such marked augmentation and may be used for a direct com parison with a standard dose response curve for approximate quantitative assays (Noble, Plunkett & Taylor 1950)

Concentrated extracts of urine, however, when injected either intraperitoneally or subcutaneously may because of their high salt content or general toxicity, result in a non specific antidiuresis This is presumably due to liberation of endogenous antidiuretic hormone or to renal circulatory changes Such fractions will not cause non specific antidiuresis if tested in the small doses required for intravenous tech niques Comparative tests of this nature have led to the belief that results obtained by using subcutaneous injections in rat assay methods are open to serious criticism

For an accurate interpretation of results, therefore it is necessary to appreciate that extracts containing antidiuretic hormone will show erroneously high activity if given subcutaneously when compared with a standard dose response curve for anti diuretic hormone (instead of a curve established using a control extract with added

1953) Again using the zinc ferrocyanide extraction we found an appreciable increase in ADS in the urine of individuals after emotional trauma induced by various practical jokes, but not after nightmares apparently involving equally severe emotional conflicts Electro convulsive therapy in mental cases is consistently associated with the excretion of ADS in the urine, whereas insulin shock therapy is not (Rechnitzer & Noble 1950)

Others have effectively used the zinc ferrocyanide method with apparent success Just recently Goldman & Luchsinger (1956) in recovery experiments were apparently somewhat surprised to obtain a yield of 109 per cent of Pitressin added to urine They obtained values indicating an average excretion of 51 mU of antidiuretic hormone over 16 hours in normal individuals In most of our experiments which have been cited the doses of extract from normal or control urines have been adjusted so that they contain only trace amounts of antidiuretic activity For every 100 c c of starting urine of the specific gravity of 1 010, the extract was concentrated to 5 ml or 5 per cent of the initial volume This was given to rats in doses of 0 5 c c /100 g i e the equivalent of 10 ml of urine/100 g body weight was administered Since the assay method used requires at least 1 0 mU /100 g body weight an antidiuretic response could not be expected unless the urine contained more than 10 mU /100 ml which would be equivalent in humans to an excretion of approximately 200 mU / day However Taylor & Jessup (1954) have studied the 24 hr excretion of antidiuretic hormone in normal persons, using extracts concentrated to 1 per cent of the starting urine volume They have reported that they could extract activity equivalent to 25–75 mU of antidiuretic hormone daily values very similar to those found by Dekanski (1951) Also, Taylor & Walker (1951) were able to show from studies on urine that one of the less dangerous but more consistently predictable perils of cigarette smoking was a stimulation of posterior lobe secretion with the liberation of 0 2 to 1 0 unit of antidiuretic hormone of which 50 mU found its way into the urine Burn & Singh Grewal (1951) have confirmed this finding and using the zinc ferrocyanide method extracted the equivalent of 75 to 100 mU from urine obtained after cigarette smoking

Many of these experimental findings have been a fitting epilogue to earlier observations that the same various stimuli led to an inhibition of water diuresis in the donor through stimulation of the neurohypophysial system The work of Verney (1946) and Gilman & Goodman (1937) on osmotic stimulation and dehydration Brun Knudsen & Raaschou (1945) on fainting and Burn Truelove & Burn (1945) on cigarette smoking may be cited In experiments of a similar nature Dr Taylor (1956) who has continued his interest in studies of posterior lobe function at the Defence Medical Research Laboratories in Toronto has been able to correlate to a high degree an inhibition of water diuresis to susceptibility to motion sickness in humans At present he is studying the excretion of antidiuretic hormone in the urine of susceptible individuals after exposure to motion The finding of antidiuretic activity in the urine in experiments of the type described have strengthened the evidence of endogenous liberation of posterior lobe hormones in such conditions, although it must be admitted that conversely it has been doubted whether the appearance of antidiuretic activity in the urine at such appropriate times favours the probability of the active urinary principles being of posterior lobe origin

Dr O G Edholm, who was studying the effects of injections of posterior pituitary extracts and of haemorrhage under various controlled conditions on certain cardio vascular responses in man In 9 cases 'Pitressin' in doses of 2 to 5 units was given in travenously The equivalent of 200 to 400 c c of urine from these individuals before injection was found to have no antidiuretic activity when extracted by the zinc ferrocyanide method Following the intravenous injection of Pitressin, however, all subjects showed extractable antidiuretic activity in the urine over the next 4 hr Quantitatively, the amount of antidiuretic activity excreted and extracted averaged approximately 13 per cent of that injected, but varied in different individuals from 4 5 to 30 per cent (Noble & Taylor, 1953) Others have obtained a 5 per cent antidiuretic activity from extracts of urine prepared by the zinc ferrocyanide method after the intravenous administration of up to 1 5 units in humans and believed that the method recovered 81 per cent of antidiuretic hormone when added to urine (Burn & Singh Grewal 1951)

Following mild dehydration in humans which follows the withholding of free fluid in the diet there is an excretion of ADS in the urine Again, it was found that the urine from hydrated subjects did not show such activity but withholding fluids for periods of 24 up to 65 hr caused an increased excretion of antidiuretic substance which could be extracted from the urine Perhaps more striking is the effect of with drawing all fluids from groups of rats and extracting the collected urine at different intervals At 24, 48 or 72 hr of dehydration a marked and increasing amount of antidiuretic substance is found in the urine From such studies on dehydration the amount of antidiuretic substance excreted can be calculated If an approximate figure of 13 per cent urinary excretion of injected posterior lobe hormone is taken from the previously described recovery experiments, then it can be estimated that during the third 24 hr period of dehydration rats secreted 'assumed antidiuretic hormone at an average rate of approximately 0 515 mU /min /kg body weight In the human cases after much less severe dehydration the maximum rate of secretion was only 0 005 mU /min /kg (Noble & Taylor, 1953)

If one can assume from the preceding evidence that the antidiuretic substance ex tracted from urine by the zinc ferrocyanide method is one of the posterior lobe hor mones then it should be possible to employ this method in a study of posterior lobe function Of the various conditions which we have studied perhaps one of the most interesting is fainting In the experiments of Dr Edholm of 14 patients from whom various amounts of blood were withdrawn and then reinfused half fainted at some stage of the experiment although this was not related to the amounts of blood with drawn Dr Taylor was able to show that, whereas the urine did not contain ap preciable amounts of ADS in specimens before fainting nor at any time in those who did not faint, there was always a considerable amount of ADS extractable from urine specimens voided after fainting Furthermore, 3 subjects who fainted from psychic causes showed ADS in urine specimens immediately after the faint, but not in samples obtained after recovery From a quantitative point of view the equivalent of 85 to 372 mU of antidiuretic hormone was extracted after fainting indicating a liberation of from 0 7 to 3 0 units by the posterior pituitary Curiously enough in dividuals who lost consciousness associated with black out during acceleration did not excrete more antidiuretic hormone in response to this reaction (Noble & Taylor

which we find with one tenth of the quantity In Grollman s & Woods curve the doses required to show antidiuresis extended from 10 to 100 mU whereas most workers under comparable conditions find a dose range of 1 to 10 mU sufficient to show maximum antidiuresis The error of the 10 per cent recovery was therefore corrected by the application of another error of a factor of times 10 giving a satisfying 100 per cent recovery We have been somewhat reluctant to publish this marked discrepancy in results which we believe invalidates accurate studies using the charcoal method especially since the method has been used by others with such success and without comment (Stein Jinks & Mirsky 1952)

Having examined the charcoal adsorption method with such devastating findings it is with some temerity and reluctance that we venture to put forward a new method of extraction for your criticism particularly since further work on it is obviously required However this method has so many features apparently in its favour that we believed it would be justified to bring it to your attention The method is essentially an extraction technique rather than one using adsorption and is based on a method which Dr Carroll had used in a different project while working in the laboratory of Sir Alexander Todd at Cambridge Urine which may be concentrated to one fifth of its original volume for ease in handling is adjusted to pH 5 It is then extracted three times with equal volumes of concentrated phenol solution saturated with water The phenol fraction contains the activity which may then be recovered from the phenol solution by the addition of ether and back extraction with water The aqueous fraction is taken to dryness and the dried residue may be extracted with absolute alcohol to remove impurities The final extract has a total solid content of approximately one seventh of the comparable extract prepared by the zinc ferrocyanide method (57 5 mg per 100 ml of urine of the specific gravity of 1 010) (Jessup Carroll & Noble 1956)

The recovery of antidiuretic hormone added to urine or saline by the phenol method has been consistently better than 85 to 90 per cent When aliquots of the same urine were extracted by this method and that using zinc ferrocyanide the recovery was always equal to or better than that found with the adsorption method The phenol method has also been applied to the urine of rats obtained during progressive dehydration Apparently the endogenously produced antidiuretic hormone when excreted can also be extracted by this method Similarly the method has been used and is particularly suited for determining the excretion of antidiuretic hormone by normal humans Unfortunately phenol also extracts the pressor substance previously referred to in normal urine which is not posterior lobe hormone Initial tests however indicate that such extracts contain only approximately one half the pressor activity of extracts made by the zinc ferrocyanide method from the same urine samples

Perhaps the most useful contribution of the phenol method may be in its application to the extraction of antidiuretic hormone from blood It is obviously beyond the scope of this paper to review this particular field but it may be mentioned that the addition of the phenol to plasma or serum acts as a protein precipitant at the same time as it extracts the posterior lobe hormone When antidiuretic hormone is added to plasma it may be recovered equally as well as from urine by the phenol method A single experiment was performed to compare the pooled blood drawn by heart

The suggested implication of neurohypophysial stimulation under conditions of altered osmotic pressure of the blood, fainting, emotional stress, electro convulsive therapy cigarette smoking, vestibular stimulation due to motion and to which should be added suckling in rabbits (Cross, 1951) and sexual intercourse in humans (Friberg, 1953), is sufficient to suggest that an endocrinologist is not entirely suited even to attempt to indicate tentatively a common factor or new nervous pathway which might ultimately allow all these various stimuli to reach the neurohypophysis rather I shall leave this to the neurophysiologists

I feel somewhat remiss for presenting so many of our results using the zinc ferro cyanide adsorption method of urine extraction, especially as some of these results may be familiar to you However, most of our experience has been with this method and it seemed necessary to review this before attempting to consider some newer studies The main drawback of the zinc ferrocyanide method is the high degree of impurity of the final extract especially in concentrated extracts such as may be necessary to determine small amounts of antidiuretic activity These impurities may lead to toxic side effects in the assay animals The publication of an apparently im proved adsorption method by Grollman & Woods in 1949 was therefore viewed with considerable interest In this procedure activated charcoal was used to adsorb the activity at pH 5 The activity was extracted from the dried charcoal precipitate by glacial acetic acid and from this it was precipitated by 10 volumes of ethanol-petroleum ether mixture The resulting product was reported to yield a 90 per cent or higher recovery of posterior lobe hormone Dr Jessup has studied this method extensively when applied to saline solutions or urine and has found that it leaves a great deal to be desired The final extract it is true contains only about 1/50 the amount of total solid (6 5 mg from 100 ml of urine of the specific gravity of 1 010) when compared with the zinc ferrocyanide product (310 mg from 100 ml of urine of the specific gravity of 1,010) but unfortunately it contains only about one tenth of the expected yield of antidiuretic activity (Jessup, Taylor & Noble, 1956)

When the charcoal adsorption method was applied to either saline or urine to which concentrations of Pitressin up to 200 mU /100 c c had been added the recovery was negligible For concentrations above 200 mU and up to 1 unit per 100 c c the recovery rate was approximately 10 per cent In experiments run in parallel to compare with the zinc ferrocyanide method, it was noted that this method extracted 80 per cent of the antidiuretic activity when Pitressin was added to urine in con centrations as low as from 10 to 100 mU per 100 c c of urine If the charcoal method is examined step by step and the residues extracted by the zinc ferrocyanide method and then assayed, Dr Jessup has found that approximately 25 per cent of the total activity was not adsorbed on the charcoal About 50 per cent of the activity re mained in the acetic acid eluate and was not precipitated by petroleum ether and alcohol Since 10 per cent only was recovered in the eluate the remaining 15 per cent was probably not eluted by acetic acid from the charcoal This discrepancy in the results obtained by us and those reported by Grollman & Woods is more appar ent than real Actually, our recoveries of 10 per cent using their method appear to be equally as inadequate as those reported by them but this is not obvious because their calculations of recovery were made from a dose response curve in rats which required 10 times the amount of antidiuretic hormone to give excretion times

experiments for the antidiuretic activity found in zinc ferrocyanide extracts of normal human urine and also for the activity extracted by the phenol method from the urine of dehydrated rats Before one concludes that the antidiuretic activity of such extracts is not due, therefore, to the antidiuretic hormone it should be mentioned that in our experience thioglycol is not effective against posterior lobe hormones in the presence of large amounts of impurities such as in crude pituitary extracts The finding remains, however, that although it is not difficult to destroy posterior lobe hormone added to urine extracts with thioglycol, it has not been possible to destroy presumed posterior pituitary hormone of endogenous origin in similar extracts In view of the evidence previously presented it would appear probable that the antidiuretic activity in urine extracts *is* of pituitary origin so that it would seem that endogenous posterior pituitary hormone may be present in the urine in some form which is protected from the action of thioglycol but is yet extractable and active on injection into the test animal

It is probably fitting, therefore, to conclude this paper with the warning that any suggestion that the antidiuretic activity obtained with any method of extraction respresents a stimulation and liberation of hormones by the neurohypophysis should be regarded as speculation until the time when chemical identification methods are practical The cautious and critical approach to the whole problem of the anti diuretic activity of urine would still seem to be justified in case erroneous conclusions as to hypophysial function are drawn which would further complicate an already difficult field of neurophysiology

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puncture from 5 normal rats under ether anaesthesia, with that removed from 5 animals dehydrated for 72 hr The extracted plasma drawn from rats by this exsanguination technique showed a marked antidiuresis on assay, and when compared with a standard of Pitressin, indicated a level of 1 2 mU per c c The animals previously dehydrated yielded a plasma containing 4 8 mU per c c under comparable conditions These values are obviously high, since they have not been corrected for the augmenting action of the serum On the other hand, following exsanguination and ether anaesthesia a value of 1 2 mU per c c for rat serum was obtained on intravenous assay by Ginsburg & Heller (1953)

The evidence which has been presented from the study of results obtained for the antidiuretic activity of urine using extraction procedures, the errors of which are recognized, strongly supports the contention that it is the antidiuretic hormone in the urine which is being extracted Thus the quantitative recovery of antidiuretic hormone added to urine the immediate appearance of antidiuretic activity in the urine following the intravenous injection of posterior pituitary extract, the increasing activity of the urine paralleling increasing dehydration, and the amounts of antidiuretic activity obtained in urine extracts after smoking or fainting are indicative that the antidiuretic hormone is being excreted Furthermore, the quantitative data which have been obtained in these experiments, using Burn s method of assay are in close agreement with those reported by others using different assay techniques

It would perhaps be convincing if one concluded at this time that the antidiuretic substance extracted from urine represented a secretion by the neurohypophysis However since this paper was designed to present some of the difficulties encountered and criticisms levelled at this experimental approach it is only fitting to record some recent findings which are perhaps at variance with the concept so far developed In general the properties of the active substance in urine extracts correspond closely to those of the pituitary antidiuretic hormone and we have been impressed with observations on the stability to heat at various pH levels It has been found repeatedly for example, that at pH 10 rapid inactivation of active extracts takes place on heating (Noble Plunkett & Taylor 1950) The use of thioglycol to inactivate antidiuretic hormone has been advocated repeatedly by van Dyke, but curiously enough, Ames & van Dyke (1951) found that sodium thioglycollate would cause an antidiuresis *per se* and was toxic if administered subcutaneously to rats However it was well tolerated if given intravenously and did not cause a complicating antidiuresis so that it proved to be an efficient inactivator of antidiuretic hormone if intravenous assay methods were used Since the assay method used in our laboratory involved the subcutaneous injection of the test extract we had not attempted to inactivate urine extracts with thioglycol until recently Rather to our surprise Dr Jessup failed to find antidiuresis or other toxic manifestations when sodium thioglycollate was given subcutaneously to fed or fasted rats of the Sprague Dawley strain This is in keeping with the earlier observations of Ralli, Raisz, Leslie Dumm & Laken 1950) On the other hand approximately 85 per cent inactivation by thioglycollate of antidiuretic hormone in saline or extracts of urine prepared by the zinc ferrocyanide or the phenol method was found by assays employing subcutaneous injection When however the activity of extracts was presumably due to endogenously produced posterior pituitary hormone no inactivation could be produced by thioglycol This has been demonstrated in single

200 c c of normal urine would be expected to contain about 5 mU of antidiuretic activity

van Dyke I was thinking of approximately 10–20 mU that would be 10 or 20 times the dose you would need for a single intravenous antidiuretic injection in the dog Such assays may be worth trying if the phenol method continues to hold promise

Our experiments with thioglycollate were performed because Ralli and her co workers (*Amer J Physiol* **163** 141 1950) reported that there are two antidiuretic substances in Pitressin one of which is active in the rat but not in the dog Her con clusions for the rat were based upon the fact that thioglycollate appeared not to inactivate the hormone causing antidiuresis in the rat Unfortunately she did not do control experiments with thioglycollate itself We found that thioglycollate is quite toxic subcutaneously in the doses Dr Ralli had employed In many cases we ob served haematuria and obvious evidence of renal damage I wonder whether you used doses as large as Ralli had employed in her experiments

Noble We used the same dose which you found to be toxic and we increased con siderably above it and to be on the safe side we also went considerably below We are at a complete loss to explain this difference in results There was no suggestion of toxicity or antidiuresis at all in the Sprague Dawley strain of rats good inhibition of posterior lobe activity by thioglycollate was found when tested against standard extracts using subcutaneous injections

Dale I do not want to complicate matters by suggesting another technique but I wonder if Dr Noble has ever tried what seems to me to be a very elegant and useful process which my late colleague Dudley used After preliminary purification and neutralization of the posterior lobe extract Dudley added sodium benzoate and then on acidification with hydrochloric acid obtained a finely crystalline precipitate of benzoic acid to which the active hormones adhered This precipitate was collected washed and dried Place in a thimble extract the benzoic acid with dry ether and you are left with a very small intensely active residue I don t know whether it would work with urine but it might be worth trying Somebody in Toronto tried it I think at a certain stage of insulin isolation but of course they eventually found better methods for that particular separation

Noble I think that we explored that method It did extract some activity but at that time we were not trying to do quantitative experiments It would be interesting to try the method you suggest

Dale If it works the benzoic acid method might have great advantages The activity is separated on a crystalline precipitate of benzoic acid which is soluble in organic solvents and these do not dissolve the active material

Cole I would like to ask Professor Noble if he has tried feeding a high sodium diet to see its effect on the excretion of antidiuretic hormone in the urine

Noble No

Eisen With regard to the nature of the antidiuretic substance in urine I should like to mention some studies carried out by Dr A A G Lewis and myself (Eisen &

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Discussion

Chairman J M Yoffey

van Dyke I feel that if Professor Noble could perform intravenous antidiuretic assays he might be more satisfied For that reason I should like to ask him how much antidiuretic activity the extract contained How many milli units were present in addition to this pressor substance?

Noble The pressor activity was actually in an extract of normal hydrated human urine, which would not have enough antidiuretic activity to show at all by our ordinary methods The 2 c c of 1 per cent extract which would be equivalent to

The effects of haemorrhage and plasma hypertonicity on the neurohypophysis

by

M GINSBURG *and* L M BROWN

Department of Pharmacology University of Bristol

IN this symposium so far we have been concerned mainly with the morphology of the neurohypophysial system the chemistry and the fate of neurohypophysial hormones Most of the papers we are to hear during the remainder of the symposium deal with the action of the hormones in regulating and controlling bodily function The hormonal control of body functions is usually achieved by a sequence of three events First a change within or on the surface of the organism affects sensory receptors which are either part of the gland or connected to it secondly, a response by the gland to stimulation alters the rate at which hormones are liberated into the circulation and thirdly cellular activity in target organs is affected by blood borne hormones

Much of our work aims at revealing the nature of the initiating stimuli and the action of the hormones for where a hormone s effect is related to a change which provokes its liberation a self correcting, physiological mechanism may be involved While there can be no doubt that the antidiuretic, milk ejecting and possibly oxytocic actions of posterior pituitary hormones participate in such mechanisms it is usually denied that the vasopressor action of the antidiuretic hormone has physiological significance This assertion is usually based on the argument that the amounts of the hormone required to affect blood vessels are much greater than are commonly believed to be liberated from the gland However it has been shown that haemorrhage provokes an antidiuretic response and so we undertook further study of this phenomenon to see firstly whether during haemorrhage there was a discharge of the antidiuretic hormone from the posterior pituitary in amounts great enough to suggest that it aided the maintenance of blood pressure and secondly if we were successful to determine how the body changes during haemorrhage affect the neurohypophysis

There are two ways in which such a problem can be approached First by study of changes in the function of a target organ in an animal subjected to manœuvres which may alter the rate of secretion of the hormone Observations of this kind were made by Rydin & Verney (1938) who found that removal of small volumes of blood from an artery (sufficient to lower blood pressure by 10 mm Hg) in water loaded dogs brought about a marked inhibition of urine flow and that this response to haemorrhage did not depend on the innervation of the kidneys or the secretion of adrenaline However in experiments in man Lewis (1953) found that removal of as much as 1 200 ml of blood by venesection did not affect water diuresis

Lewis, *Lancet* ii, 361, 1954) with the urines of post operative patients collected during the twenty four hours following surgical procedures of various extent Acidified, filtered urines, kept in the deep freeze and diluted 5-20 times, were assayed by in travenous route in ethanol anaesthetized rats and in a well trained dog with established experimental diabetes insipidus The antidiuretic activities found ranged from 0 05 to 2 5 mU /ml Our reasons for believing that this was due to posterior pituitary hormones were the following the responses in the two species of test animals were mostly of the same order of magnitude in terms of Pitressin activity In the dog, the antidiuretic responses occurred without changes in the endogenous creatinine excretion and could be ascribed to tubular water reabsorption In rats the slopes of the log dose response curves of urinary activity and Pitressin were highly similar Urinary activity was destroyed by boiling in alkali and by contact with sodium thioglycollate

I can confirm Professor Noble s observations that sodium thioglycollate occasion ally does not destroy posterior pituitary factors We found that destruction is more successful when the salt is freshly prepared and contact is maintained for at least 30 minutes Some rats showed, after one or more intravenous injection containing sodium thioglycollate, a greatly reduced responsiveness to Pitressin

Reiss Professor Noble did you test your extracts for the presence of anterior pituitary hormones?

Noble The phenol extraction method would extract certain of the anterior pituitary hormones but we did not test for them as a routine It is curious that preliminary tests indicate that it will apparently extract FSH from post menopausal urine, but not chorionic gonadotrophin

Chalmers Lewis and I some time ago observed antidiuresis after fainting and we found that the duration of the antidiuresis was matched by intravenous injections of Pitressin of the order of 0 5 to 2 0 U which I think agrees very well with what Professor Noble found

increased so that in the fifth millilitre it was at least 10 times greater than that of the first millilitre withdrawn The activity in blood coming from the head was 4-6 times greater than that of arterial blood and the absolute difference between external jugular and arterial blood antidiuretic activity increased from 0 2 mU /ml in the first millilitre to 3 0 mU /ml in the fifth millilitre of blood withdrawn The antidiuretic substance must therefore enter the circulation in the head Later experiments showed that this substance could not be detected in the blood of neuro hypophysectomized rats and that the antidiuretic activity was abolished by treatment with thioglycollate so it may be concluded that posterior pituitary antidiuretic hormone was liberated in increased amounts during haemorrhage

It has been suggested that the antidiuretic response to haemorrhage is a con sequence of emotional disturbance, but since in the present experiments the blood was withdrawn from anaesthetized animals it is improbable that emotional stimuli were involved

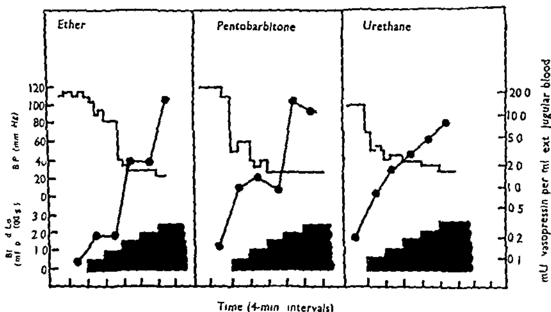


Figure 2

Effect of slow haemorrhage upon blood pressure and antidiuretic activity in external jugular blood in rats anaesthetized with ether pentobarbitone and urethane 0 5 ml blood/100 g was withdrawn at 4 min intervals

●—● mU vasopressin/ml blood

—— blood pressure mm Hg

In Fig 2 the results are shown of typical experiments in which blood was with drawn more slowly from rats anaesthetized with ether pentobarbitone and urethane Blood 0 5 ml per 100 g body weight was withdrawn at 4 min intervals from an external jugular vein and when a sample of blood was taken for antidiuretic assay an equal volume of rat blood was simultaneously returned to the animal via a femoral

The second approach, which we have adopted, is to measure the changes in the amounts of the hormone in body fluids. Noble & Taylor (1953) estimated the antidiuretic hormone content in urine in men after blood withdrawal; they found antidiuretic activity in urine of only those subjects who fainted during blood withdrawal and none in the urine of subjects who remained conscious.

In the experiments I am about to describe we have followed the changes in antidiuretic activity in blood during haemorrhage. A disadvantage inherent in this type of experiment is that the effect is observed of only that fraction of the liberated hormone which is present in a sample of blood and therefore only great changes in hormone secretion can be detected. On the other hand, the demonstration of increased amounts of the hormone in blood gives the most direct and unequivocable evidence of increased hormone secretion.

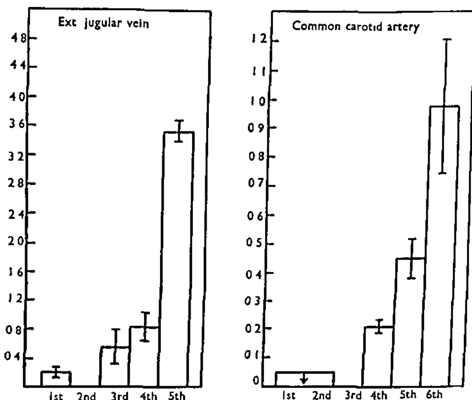


Figure 1

Effect of haemorrhage in rats anaesthetized with ether on the antidiuretic potency of external jugular and common carotid blood. The interval between the withdrawal of each ml was 90 sec.
Ordinate: Antidiuretic potency of blood (mU vasopressin/ml.)
Abscissa: ml. of blood.
(Ginsburg & Heller 1953)

Figure 1 shows the antidiuretic potency of successive millilitres of blood withdrawn at 90 sec. intervals from a common carotid artery or an external jugular vein in rats anaesthetized with ether. The antidiuretic potency of the blood from both sources

detected in the first sample of external jugular blood In all experiments with ethanol anaesthesia, the antidiuretic potency of the blood increased during haemorrhage, reaching 5–10 mU vasopressin per ml Three experiments were performed on rats anaesthetized with chloralose, in two, no antidiuretic activity (i e less then 200 μU / ml) could be detected in the external jugular blood even after removal of 3 0 ml blood/100 g body weight In the other rat (the experiment illustrated), the highest antidiuretic activity was less than 2 0 mU /ml blood The blood pressure fell by 20 mm Hg or less each time blood was withdrawn from rats anaesthetized with chloralose whereas with other anaesthetics sooner or later, the blood pressure fell suddenly by 40 mm Hg or more, and the increase in blood antidiuretic activity was associated with this fall in blood pressure It is possible therefore that the lower activity in blood during haemorrhage in chloralose anaesthesia is due to an altered pattern of blood pressure change rather than a specific effect upon the neurohypophysial system or peripheral receptors linked to that system

The antidiuretic activity of blood during haemorrhage in ethanol anaesthesia was only slightly lower than that found in experiments when ether pentobarbitone or urethane were used Ethanol can prevent the release of antidiuretic hormone in other circumstances but it does seem that the description of ethanol anaesthesia as functional neurohypophysectomy (Dicker 1953) is not justified in this connexion

Figure 4 shows the changes in antidiuretic activity of external jugular blood following a single rapid removal of approximately 40 per cent of the blood volume in a rat anaesthetized with pentobarbitone 5 5 ml of blood was withdrawn in 2 min from a femoral artery and the artery was then connected to a blood reservoir in which the blood level was 44 5 cm (equivalent to approx 36 mm Hg) above the animal s heart During the subsequent 25 min a further 0 5 ml of blood transfused out of the animal but the blood pressure did not change The antidiuretic activity in a sample of external jugular blood taken 4 min after the removal of blood was 25 times greater than that before haemorrhage The antidiuretic hormone concentration then fell rapidly and 12 min later was of the same order as that found in blood before haemorrhage This fall was followed by a second rise in the blood antidiuretic activity The essential features are similar to those of the slow haemorrhage experiments The liberation of antidiuretic hormone in the first place followed closely upon the fall in blood pressure and there was a second increase in the hormone concentration in the blood (in this case) after 20 min of hypotension

These findings indicate the liberation of very large amounts of antidiuretic hormone from the pituitary during haemorrhage and that the discharge of the hormone is more closely related to the fall in blood pressure than to changes in blood volume This may of course apply only to the massive discharge of the hormone under our experimental conditions Concentrations of antidiuretic hormone in the venous effluent from the head of the order of 10–20 mU /ml blood are certainly high enough to suggest that the hormone could have a sustaining effect on blood pressure during haemorrhage This conclusion is supported by the findings of Frieden & Kellar (1954) who measured the volume of blood which it is necessary to remove from dogs in order to lower the blood pressure to 70 mm Hg and found that in dogs with experimental diabetes insipidus this volume of blood was significantly less than in normal animals It has been suggested that the liberation of antidiuretic

vein The blood pressure was recorded from a femoral artery In these experiments we observed up to 100 fold increases in the antidiuretic potency of external jugular blood Before haemorrhage in the first sample the antidiuretic activity was 0 1–0 3 mU vasopressin/ml blood The first big increase in antidiuretic activity (about 5–10 fold) seemed to occur after the first pronounced fall in blood pressure, i e a *fall of 50 mm Hg or more In some experiments, the blood pressure fell and the* antidiuretic activity rose after the first haemorrhage, but in others neither change occurred until after three withdrawals of blood This was followed by a continuous increase in the antidiuretic activity of the blood or the activity remained unchanged until 8–12 min after the first increase, when it again rose abruptly In experiments with these anaesthetics (ether, pentobarbitone and urethane) concentrations equivalent to 10–20 mU vasopressin per ml blood were usually found after 5 haemorrhages and the highest concentration we recorded was 26 mU /ml blood

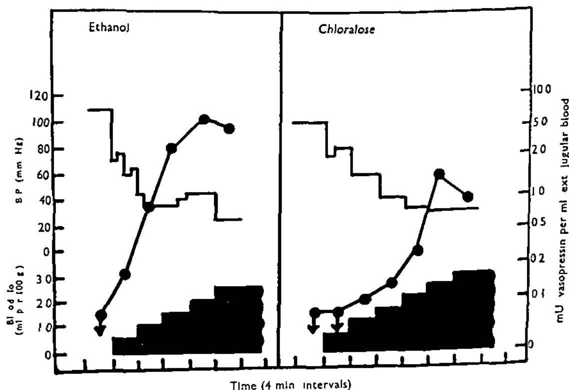


Figure 3

Effect of slow haemorrhage upon blood pressure and antidiuretic activity in external jugular blood in rats anaesthetized with (*a*) ethanol and (*b*) chloralose 0 5 ml blood/100 g was withdrawn at 4 min intervals
●—● mU vasopressin /ml blood
——— blood pressure mm Hg

Figure 3 shows results of similar experiments in rats anaesthetized with ethanol and chloralose With both of these anaesthetics no antidiuretic activity could be

rats in which the carotid sinuses were denervated in addition to bilateral vagotomy, the amounts of antidiuretic hormone were lower than those found in rats with these nerves intact However in these experiments, every time blood was withdrawn, the blood pressure fell in steps of less than 40 mm and the characteristic sudden collapse of blood pressure was not observed It is therefore not possible to conclude that in this experiment we had destroyed a specific nervous pathway through which changes in blood pressure are transmitted to the pituitary since the pattern of blood pressure change during haemorrhage had been altered However, the results obtained with vagotomized rats may be peculiar to this species Blood Kosman & d Amour (1955) have shown recently that although pulmonary depressor reflexes can be elicited in rats the responses are not abolished (as in other species) by section of the vagus nerves

A connexion between stretch receptors in the left auricle and pulmonary veins with urine flow has been demonstrated by J P Henry O Gauer and their co workers (Gauer Henry, Sieker & Wendt 1954 Henry Gauer & Reeves 1956 Henry & Pearce 1956) If these changes in urine flow which are associated with the state of filling of intrathoracic blood vessels are indeed determined by the neurohypophysial antidiuretic hormone such receptors could be involved in the release of the hormone during haemorrhage The present experiments have not given decisive information on this point, possibly because of the peculiarity of the rat mentioned above However a few years ago Professor Heller and I (Ginsburg & Heller 1953) found that blood taken by heart puncture from anaesthetized rats had greater antidiuretic activity than could be accounted for by the activities in venous blood We thought then that this might have been due to the release of antidiuretic hormone by puncture of the myocardium or alterations of the pressures within the intrathoracic circulation

These findings of a connexion between intrathoracic stretch receptors and urine flow are not entirely consistent with some older observations For example electrical stimulation of the central vagus liberates hormones from the neurohypophysis while activation of the intrathoracic stretch receptors which increases impulse activity in vagal fibres, has what would appear to be the opposite effect—it induces a diuresis Indeed at present there is little or no evidence for the view that the changes in urine flow associated with these stretch receptors are determined by posterior pituitary antidiuretic hormone—although an alternative might be hard to find

After we had shown that during haemorrhage in rats large amounts of antidiuretic hormone are liberated from the posterior pituitary we investigated the effect of haemorrhage upon vasopressin and oxytocin contents of the neurohypophysis The oxytocic and vasopressor activities in extracts of glands from rats anaesthetized with ether ethanol and chloralose were not different from those from unanaesthetized rats which had been killed by decapitation and slow haemorrhage of the anaesthetized rats did not affect the hormone contents in the glands The ratio of pressor to oxytocic activity in all extracts was not significantly different from 1 0 Figure 5 shows the results of similar experiments in rats anaesthetized with pento barbitone and urethane In the extracts of glands from rats anaesthetized with urethane, the vasopressin content was less and the oxytocin content was more than in those from unanaesthetized rats and the ratio of pressor to oxytocic activity was of the order of 0 5 again, bleeding did not affect the activities of the gland extracts

hormone in response to a fall in blood pressure might account for the antidiuresis associated with syncope and with vomiting (Andersson & Larson, 1954) Administration of histamine and veratrum alkaloids also provoke the liberation of antidiuretic hormone and these too may be responses to the fall in blood pressure

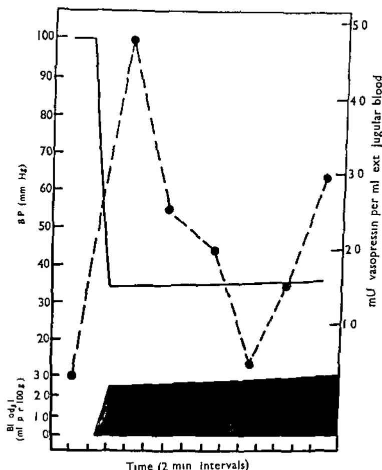


Figure 4

Effect of rapid haemorrhage on blood pressure and antidiuretic activity in external jugular blood 5 5 ml blood was withdrawn in two minutes and the blood pressure maintained at 33–37 mm Hg
● ● mU vasopressin/ml blood
——— blood pressure mm Hg

In view of the evidence that following electrical stimulation of the vagus nerve there is liberation of posterior pituitary hormones (Chang Chia Huang & Lim 1939, Andersson 1951) an obvious way in which this response could be mediated is through an increase in chemoreceptor or baroreceptor impulse activity in afferent vagal fibres However in experiments on rats in which both vagi had been divided in the neck the increase in antidiuretic activity in blood during haemorrhage was not prevented and was again associated with a sharp fall in blood pressure During haemorrhage in

the posterior lobe so that the hormones are transferred in increased amounts from the gland into the circulation and *trophic* stimuli which increase the amounts of biologically active material in the gland Ames & van Dyke (1950) found an increased vasopressin content in the posterior pituitaries of rats which had been deprived of fluid for 3 days, but in most investigations the hormone content of the neurohypophysis has been found to decrease under conditions or after treatment which would increase liberation of hormones from the gland However close examination of some of these results leads to the conclusion that trophic stimulation of the neurohypophysial hypothalamic system also occurred The normal rate of antidiuretic hormone secretion has been estimated in dogs to be of the order of 1–5 mU /hr (Shannon 1942) and 0 3–1 2 mU /hr in rats (Dicker 1954) assuming that the vasopressin content of the posterior lobe does not vary greatly from day to day this rate must be approximately equal to that at which the hormone store is normally replenished In Hild & Zetler s (1953) experiments after restoration of access to water in dogs which had been thirsted for 14 days, the vasopressor potency of neurohypophyses increased at a rate of 2 7 units/day i e the rate of repletion was 20–100 times greater than normal More striking perhaps, are the results of Dexter, Stoner & Green (1954) who showed that following depletion by ATP injection the antidiuretic activity of rat posterior pituitaries was restored by over 200 mU (one third of the content of a normal gland) in 30 min i e 330–1 300 times faster than the normal rate Similarly Rothballer (1953) found that painful stimuli in rats caused almost complete depletion of neurosecretory substance in the neurohypophysis within a few minutes, after 2 hr the normal content of neurosecretory material was restored Since the activity in the hypothalamus is small compared with that in the pituitary repletion at such rates cannot be explained solely on the basis of increased rate of flow of the axoplasm current (Scharrer & Scharrer 1954) Such stimuli must also increase the rate at which active material is formed in hypothalamic nuclei The amounts of active substances in the neurohypophysis are determined by the rate of repletion as well as by the rate of hormone release and the turnover of hormones by the gland may be independent of the level at which they are stored Change in the hormone content of the gland is therefore not a reliable index of secretion

The liberation of antidiuretic hormone during haemorrhage appeared to occur in two stages The first discharge of the hormone was associated with a fall in blood pressure and the second one occurred after 10–20 min of sustained hypotension We thought that the second increase in the concentration of antidiuretic hormone in blood might be due to the liberation of substances such as ATP or ferritin which could stimulate the further release of antidiuretic hormone

To test this hypothesis blood was taken from a rat during sustained hypotension and its antidiuretic effect when given into an internal carotid artery was compared with its effect after intravenous administration We found that the antidiuretic effect was the same when the blood was given by either route However when 0 5 ml of 4 5 per cent NaCl solution was injected into the carotid there was no inhibition of the diuresis This disturbing observation demanded further investigation

The injections were given to unanaesthetized rats through an exteriorized polyethylene cannula inserted into an external carotid artery between the origins of the occipital and superior thyroid arteries The tip of the cannula was placed close

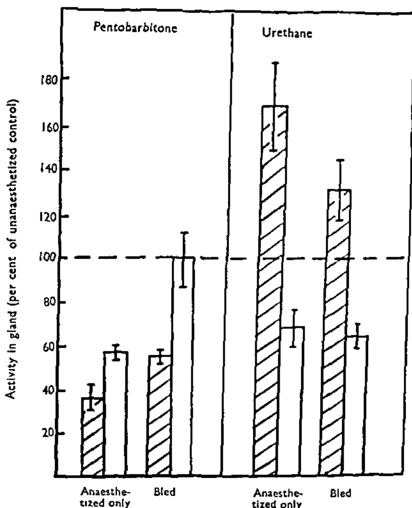


Figure 5

Effect of anaesthesia with pentobarbitone and urethane and the effect of haemorrhage on the oxytocin and vasopress n contents of rat neurohypophyses

oxytocin

vasopressin

In rats anaesthetized with pentobarbitone the oxytocin and vasopressin contents of the gland extracts were less than in the unanaesthetized controls with greater depletion of oxytocin so that the ratio of pressor to oxytocic activity was now of the order of 2 0 In gland extracts from rats which had been bled under pentobarbitone anaesthesia, both the oxytocin and vasopressin contents were significantly greater than in glands from animals which were only anaesthetized although the ratio of pressor to oxytocic activity remained at about 2 0 Since haemorrhage under these conditions undoubtedly causes liberation of antidiuretic hormone into the blood, this finding can be explained only by increased repletion of the hormones stored in the gland It may therefore be necessary to distinguish between *tropic* stimuli which affect

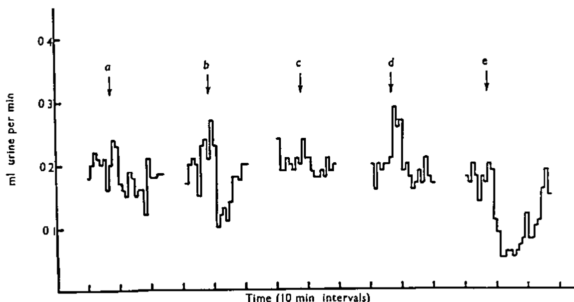


Figure 7

Effect of slow injection (30 sec.) of hypertonic NaCl solution on urine flow in unanaesthetized rat

(*a*) 30 μU vasopressin in 0·3 ml 0·9 per cent NaCl i.v.
(*b*) 30 μU vasopressin in 0·3 ml 4·5 per cent NaCl i.v.
(*c*) 0·3 ml 0·9 per cent NaCl into left internal carotid
(*d*) 0·3 ml 4·5 per cent NaCl into left internal carotid
(*e*) 60 μU vasopressin in 0·3 ml 4·5 per cent NaCl i.v.

When hypertonic solutions were injected more slowly the interference with administered Pitressin was absent or not prominent. Figure 7 shows the results of an experiment in which the water load was maintained by water administration by mouth. When 30 or 60 μU of Pitressin were given in 4·5 per cent NaCl solution and the injection given over 30 sec. there was no interference with the antidiuretic responses but again when a hypertonic NaCl solution was injected into an internal carotid a brief enhancement of the diuresis occurred without any indication of an antidiuresis. In this experiment the absence of an antidiuretic response cannot be attributed to interference with the effect of the hormone on the kidney.

These findings in rats are not in agreement with those of Verney (1947) in his experiments on dogs. Verney did not investigate the effect of rapid intravenous injection of hypertonic solutions on the antidiuretic responses to injected vasopressin in his dogs but judging from the effects he obtained following intracarotid injection of hypertonic solutions the amounts and rates of his injections did not interfere with antidiuretic responses.

The effect of continuous intracarotid infusion of hypertonic NaCl solution is shown on Fig. 8. The urine flow increased during intracarotid infusion of 1·8 per cent NaCl. When the hypertonic salt solution was infused intravenously together with Pitressin the antidiuretic effect of the Pitressin was masked so that the urine flow recovered even while the Pitressin administration continued but there was a definite antidiuretic effect during the first ten minutes of infusion. In this experiment NaCl

to the carotid bifurcation and its wall blocked the origin of the occipital artery. Injections made into this cannula are driven into the internal carotid by the force of the arterial pressure. The cannulae were inserted, under ether anaesthesia, 4–5 hr before the experiment.

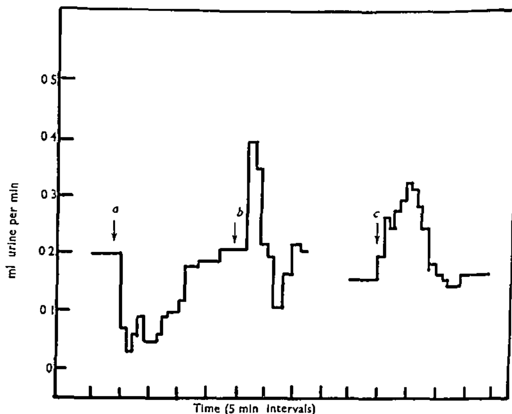


Figure 6

Effect of rapid injections (5 sec.) of hypertonic NaCl solution on urine flow in unanaesthetized rat.
(*a*) 300 μU vasopressin in 0.4 ml 0.9 per cent NaCl i.v.
(*b*) 300 μU vasopressin in 0.4 ml 3.6 per cent NaCl i.v.
(*c*) 0.4 ml 3.6 per cent NaCl into right internal carotid.

Figure 6 shows the results of one of our first experiments. In a rat with cannulae in the bladder, external jugular vein and an external carotid artery, a high water load was maintained by intravenous infusion of a 1 per cent. solution of dextrose. Intravenous injection of 300 μU *of Pitressin in 0.9 per cent. NaCl given in 5 sec. had a* typical antidiuretic effect lasting 10–15 min. When the same dose of Pitressin was given in 3.6 per cent. NaCl solution, the antidiuretic effect was masked. During the two minutes following the injection, the urine flow was almost doubled and thereafter fell to slightly below the initial rate to which it returned about 10 min. after the injection. When hypertonic NaCl solution was given into the right internal carotid the urine flow increased for about 10 min. without any indication of an antidiuretic effect.

was infused at the rate of 12 5 mg /kg /min increasing the osmotic pressure of the internal carotid blood by 15 5 per cent For comparison when Verney, in one of his experiments infused a hypertonic NaCl solution into a common carotid in a dog giving 11 0 mg NaCl/kg /min and increasing the osmotic pressure of the blood by 18 0 per cent profound and long lasting inhibition of urine flow was produced

Figure 9 shows that results essentially similar to those obtained during infusion of NaCl solution were also obtained during intracarotid infusion of hypertonic solutions of sucrose In this experiment an isotonic sucrose solution was infused into the right internal carotid for 10 min causing a slight enhancement of the diuresis This was followed by an intracarotid infusion of 31 8 per cent sucrose at the same rate which we calculated would increase the osmotic pressure of the carotid blood by 14 5 per cent The rate of urine flow was more than doubled The third intracarotid infusion given in this experiment was also 31 8 per cent sucrose but the infusion rate was reduced so that the animal received the same total quantity of sucrose as during the first isotonic infusion The increase in osmotic pressure here was 3 0 per cent Again there was a slight enhancement of the diuresis very similar to that obtained during the infusion of isotonic sucrose

These findings appear to be in direct contradiction to the findings in dogs by Verney (1947) Ames Moore & van Dyke (1950) and Abrahams & Pickford (1954) At first we considered the possibility that the injections were not in fact entering the internal carotid artery and the cerebral circulation During intracarotid injection or infusion of strongly hypertonic solutions the rats showed signs of excitement and dis comfort rotation of the head and jaw movements reminiscent of chewing were frequently observed These effects did not occur after intravenous administration of hypertonic solution About 2–3 seconds after intracarotid injection of Evans Blue in albino rats, the retina was seen to be momentarily suffused with blue dye The best evidence that the injections entered the internal carotid was obtained by injecting ^{24}Na 0 3 ml of a 0 9 per cent NaCl solution containing 200 micro curies of ^{24}Na was injected into an internal carotid or an external jugular vein in 30 sec immediately after the completion of the injection the rat was decapitated The brain was divided medially and the distribution of radio activity was determined in each half When ^{24}Na was injected as we believed into the right internal carotid artery the radio activity on the right side of the brain was greater than on the left side The biggest differences were found in the temporal lobes and the least in the medulla When in travenous injections were given much smaller amounts of radio sodium were found in the brain and there were no marked differences between the right and left sides Whatever else these results mean they do indicate that injections given by our technique do enter the internal carotid artery and so reach the brain before general mixing with the circulating blood

The explanation of our findings is a formidable task and we have always considered that they may be artefacts without physiological significance For example it is possible that osmoreceptors in the bed of the internal carotid artery are not respon sive to stimulation under the conditions of our experiments The animals had been operated under ether anaesthesia only 4–5 hr before the experiment and this could have altered the sensitivity of the osmoreceptors When the animals were water loaded it was noted, as might be expected that the delay before the onset of water

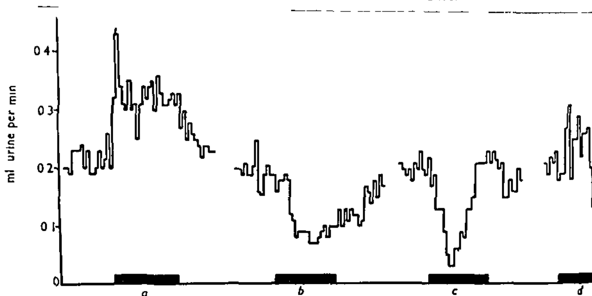


Figure 8

Effect of continuous infusion of hypertonic NaCl solution on urine flow in unanaesthetized rat

(*a*) 1 8 per cent NaCl at 0 2 ml /min into right internal carotid
(*b*) 40 μU vasopressin in 0 2 ml 1 per cent dextrose/min into left external jugular vein
(*c*) 40 μU vasopressin in 0 2 ml 1 8 per cent NaCl/min into left external jugular vein
(*d*) 1 8 per cent NaCl at 0 2 ml /min into left external jugular vein

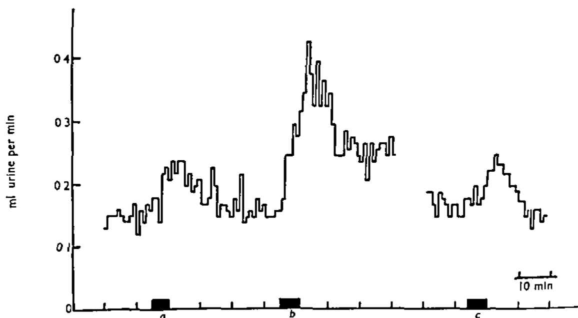


Figure 9

Effect of continuous infusion of hypertonic sucrose solution on urine flow in unanaesthetized rat

(*a*) 10 6 per cent sucrose at 0 17 ml /min into right internal carotid
(*b*) 31 8 per cent sucrose at 0 17 ml /min into right internal carotid
(*c*) 31 8 per cent sucrose at 0 06 ml /min into right internal carotid

water loading Such observations cannot be explained by the osmoreceptor theory and it has been proposed that there are 'volume receptors which are sensitive to changes in the circulating blood volume Secretion of hypotonic urine can be induced by procedures which increase the effective circulating blood volume, while anti diuretic effects are produced when the effective circulating blood volume is reduced, without increasing the plasma osmotic pressure It was at first difficult to conceive the nature of these volume receptors but it is possible that they may be the in trathoracic stretch receptors described by Henry and his colleagues (Henry, Gauer & Reeves 1956, Henry & Pearce (1956) They point out that volume changes could be sensed by some part of the circulatory system with greater than average distensi bility and suggested that the low pressure parts of the intrathoracic circulation could play such a role Many of the procedures which, without changes in tonicity, result in increased urine flow and for which volume receptors had been postulated, lead to engorgement of the intrathoracic vessels and conversely that procedures which cause antidiuresis lead to reduced filling of these vessels However as I pointed out earlier there is as yet no evidence that the changes in urine secretion associated with activation of these stretch receptors are dependent upon the antidiuretic hormone

Following the injection of hypertonic solutions according to one theory the increase in plasma osmotic pressure will tend to increase antidiuretic hormone output while according to another view the expansion of the extracellular fluid volume will affect the intrathoracic receptors in such a way as to enhance diuresis In the physiological counterpart—water deprivation—this paradox is not encountered the plasma osmotic pressure will tend to increase without a simultaneous expansion of the extracellular fluid volume

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diuresis was greater than normal, indicating that antidiuretic hormone had been liberated during the anaesthesia and operation The antidiuretic hormone content of the posterior pituitaries of such operated animals was not less than normal, at the time of the experiment but it is possible that the hormone is not in a physical state or position in a gland which has been recently depleted to permit its discharge into the circulation

A second possibility concerns the concomitant secretion of oxytocin Abrahams & Pickford (1954), found that following intracarotid injection of hypertonic NaCl in dogs the amounts of antidiuretic hormone liberated were only about one thirtieth of the oxytocin simultaneously released Fraser (1937, 1942) has shown that oxytocin preparations increase the diuresis rate in water loaded rats (but not in dogs) and that oxytocin (given in excess) can prevent the antidiuretic response to administered vasopressin If, after osmotic stimulation in rats there is preponderance of the oxytocic principle in the secretion from the pituitary then it is possible that an antidiuretic response may be obscured

Other effects due to the injection of hypertonic solution in water loaded rats may prevent the secretion of antidiuretic hormone or prevent the hormone from affecting urine flow For example as we have shown, intravenous administration of hypertonic solutions interferes with antidiuretic responses but this alone cannot account for the failure of the intracarotid injections to cause antidiuresis However in experiments of this type elevation of osmotic pressure in one arterial bed and not in others is entirely contrived by the experimental situation and could not occur normally It must be shown, I think that increases in the osmotic pressure of renal arterial blood do not impair antidiuretic responses to identical and simultaneous increases in carotid blood osmotic pressure Such conditions must come perilously close to those which have been found by del Greco & de Wardener (1956) to result in the production of a diuresis of hypotonic urine which is not inhibited by Pitressin Such a diuresis may be responsible for the interference with antidiuretic responses which we observed during infusion of hypertonic solutions although it is just as likely that increases in the tonicity of the urine and changes in GFR are involved

The experiments of Chambers Melville Hare & Hare (1945) in which they showed that intravenous injections of very large amounts of NaCl in hypertonic solution cause greater increases in urine volume in diabetes insipidus dogs than in normal dogs go some way to meeting these criticisms However, in experiments of this type in normal animals or human subjects there seems to be an unduly great delay between the administration of hypertonic solutions and the onset of antidiuresis An osmotic diuresis induced by the salt does not seem to be the sole explanation since for example in experiments by Kleeman Rubini, Lamdin & Epstein (1955) the free water clearance was not reduced in water loaded subjects until 30 min after cessation of an intravenous infusion of 5 per cent NaCl solution of 1 hr duration

During the last five years numerous investigators have shown that diuresis of a hypotonic urine is not solely dependent on the dilution of electrolytes in plasma and may be induced even by administration of slightly hypertonic solutions For example Borst and his co workers (Blombert Gerbandy, Molhuysen de Vries & Borst 1951) found that the first effect of infusing slightly hypertonic solutions into recumbent subjects was the production of a hypotonic urine similar to that obtained after simple

Jewell Dr Ginsburg has given us many possible reasons why the results he got should differ from those of Verney and it is quite clear there are an enormous number of factors involved here, making it very difficult to find one s way amongst them A few points did occur to me while Dr Ginsburg was talking The first relates to the blood distribution We ought to be quite sure that the blood was reaching the brain There is great variation between animals in this regard In the dog, for example usually only the most anterior part of the hypothalamus receives carotid blood and the rest of the brain posterior to it is supplied by the vertebrals In the goat on the other hand, as Dr Andersson and I have recently discovered, the whole of the brain is supplied by blood from the carotids I believe the rat has quite a good vertebral supply so that there might be something here that should be looked at further although your experiments with radio active sodium did suggest that carotid blood was reaching the hypothalamus and hence this argument is met I wonder whether the dextrose may be responsible for the discrepancy It will be remembered that Verney found that dextrose failed to release hormone when used to raise the osmotic pressure of the blood and I believe he did entertain the idea that there may actually be an interference with the output of the hormone or its release directly due in some way to the dextrose or to the insulin released by it in addition to the dextrose being an ineffective osmotic stimulant Could this factor affect your experiments? Those unanaesthetized rats, I suppose must be restrained in some way I wonder if emotional disturbance and release of adrenaline might be interfering at the hypo thalamic or renal level In experiments on the dog using intracarotid infusions of sodium chloride I think you could get a masking of the effect of the antidiuretic hormone when the quantities released are very small by a direct osmotic or filtration effect upon the kidney Sometimes during the period of infusion of hypertonic solu tions into the carotid the urine flow rises during the ten minutes infusion period and antidiuresis only appears if at all after you stop the infusion Under your experi mental conditions these factors might be of greater importance Finally there is the question of the water load In the experiments which I showed the other afternoon when Dr Andersson and I had kept a dog hydrated for ten days we didn t actually do that to look at the neurosecretory material for we are novices in the study of neurosecretion We really wanted to see whether the responses to intracarotid infusion of hypertonic solutions were the same before and after this period of hydration We found when we gave a previously effective intracarotid injection that there was no response after the prolonged period of hydration

Ginsburg To deal first with Dr Lloyd s point I think that the conditions of his ex periments are so much different from ours that little can be gained by comparing them at the present moment To turn to Dr Jewell s point about the distribution of the injection in the brain we did find more radio sodium in the right hypothalamus than in the left side when the injection was made into the right internal carotid artery also I think that Daniel Dawes & Pritchard (*Philos Trans* B **237** 173 1953) have shown that in the rat the anastomotic arteries are absent and thus internal caro tid injections in rats have a better chance of reaching intracranial receptors than in dogs Rats produce urine rather quickly and after oral administration of water it is very difficult to keep a sufficient water load in the animal That is why we used

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Discussion

Chairman G W Harris

Lloyd We have a few experiments on the injection of hypertonic solution in rats which were done quite differently from those of Dr Ginsburg These were in animals which had been dehydrated for 48 hr With moderate dehydration of this degree we found an increase in antidiuretic activity in the hypothalamus When the animals were given hypertonic saline very rapidly—so rapidly indeed that a number had convulsions—we found a decrease in the antidiuretic hormone content of the neurohypophysis I wonder if it was a question of the animals being anaesthetized or a question of the very rapid injection not permitting mixing with the rest of the circulating blood or else acting as a non specific stress

of Pitressin is measured by the rate of urine flow, because unless the rate of solute output is known it is impossible to deduce whether a change in urine flow is due to a change in the level of circulating antidiuretic hormone or to a change in solute output Usually these tests are done with the understanding that solute output remains unchanged If this assumption is false then changes in urine flow cannot be used as an indication of posterior pituitary hormone activity It is then necessary to measure both the solute output and the concentration of the urine over a range of the former which is the same both before and after the experimental situation under study

Sloper Dr Ginsburg s suggestion that haemorrhage may provoke the liberation of large quantities of vasopressin is of great interest to me because it may explain a curious finding which Dr Adams and I have made in the human hypothalamus after hypophysectomy Now it is known that in a wide variety of animals neurosecretory material accumulates above the cut pituitary stalk This accumulation is rarely marked before about the fourth day after operation—Dr Stutinsky has a particularly wide experience in this field—but in the human only 32 hr after hypophysectomy we have found a marked accumulation of neurosecretory material above the cut pituitary stalk Now in man there is a good deal of haemorrhage during the hypophysectomy and I wonder if the severity of this haemorrhage could account for the apparent rapidity of the mobilization of this neurosecretory material

O Connor I would like to refer to the earlier part of Dr Ginsburg s paper—that dealing with the effects of haemorrhage and refer to experiments on the dog Rydin & Verney (*Quart J exp Physiol* **27** 343 1938) found that bleeding about 40 ml from the femoral artery produced inhibition of water diuresis That was in dogs in which the kidneys were denervated involving a partial destruction of the sympathetic nervous system Certainly the splanchnic nerves had been cut and I do not remember whether also the lumbar sympathetic chains were also removed Some years ago I tried to experiment on the effect of haemorrhage on water diuresis in dogs with sympathetic system intact and found that bleeding up to 150 ml from the jugular vein had no effect on water diuresis I have not gone any further than this as there seemed to be no problem to investigate

I can also say that occlusion of both carotid arteries in the conscious dog does not produce an inhibition of water diuresis—rather in fact a small increase in urine flow—so that it appears that release of antidiuretic hormone cannot be mediated through the carotid sinus baroreceptors

Ginsburg I wonder whether withdrawal of even as much as 100 ml of blood from a vein in a conscious dog would have much effect upon blood pressure

O Connor I don t know anything about the change of blood pressure the pulse rate fell rather than increased

Heller When Dr Ginsburg removed relatively small volumes of venous blood one or two millilitres in rats he couldn t show any increase of antidiuretic activity in the blood He had to remove quite considerable volumes which are not comparable to

dextrose infusions However, we did a number of experiments with water loading by mouth which showed that the dextrose was not interfering, we used water loads ranging from 2–8 per cent of the body weight and the results obtained didn't seem to depend on the water load The animals were not restrained in any way, they were placed in wooden boxes with Perspex lids which do not quite cover the whole of the box top so that there is a little slit at one side, the tubes connected to the various cannulae in the animal pass out through this slit and the rats can turn round and move about inside the box as they wish I was very interested to hear, however, that in dogs you have occasionally obtained results rather like ours

Pickford I thought that was a very fascinating account and if you really have found a use for vasopressin *per se* it is really important and interesting Dr Jewell has already made some of the points that I had in mind and a number of others as well There is one on which I have personal experience and that is on the question of anaesthetizing animals only a few hours before making observations When I was wanting to make observations on water diuresis under light choralose anaesthesia with the pituitary exposed so that I could manipulate it, first of all I exposed the pituitary under ether in the morning and then I proceeded to make the observations in the afternoon, but I found that urine secretion and the responses of the pituitary simply were not normal within a few hours of administering ether, so I then started doing my preliminary operation on the previous day leaving a good twentyfour hours for recovery I think your suggestion correct that ether anaesthesia may have something to do with the difference between your results and those obtained in dogs The other question concerns these intrathoracic receptors which are probably baroreceptors We have a small bit of evidence that they are concerned in antidiuresis, it is based on the results of injecting 5 hydroxytryptamine into different parts of the circulation If you inject 5 hydroxytryptamine into a fore limb vein so that it reaches the intra thoracic viscera first, then it produces its greatest antidiuretic effect

Ginsburg It is always possible that the recent operation and anaesthesia interfered with the responses of the pituitary, but we found as you could see, that at least the urine flow and the responses to injected Pitressin were not abnormal in the recently operated animals

Cole I would like to mention two points from my own work which may have some interest in connexion with Dr Ginsburg s paper I have been using rats with an indwelling cannula in the external jugular vein and gave them constant infusions of isotonic Krebs bicarbonate solution over a period of eight hours These animals develop a very marked increase of urine flow and sodium excretion This increased urine flow is slightly reduced by giving Pitressin intravenously in doses up to 200 mU This to my mind, tends to suggest that a diuresis caused by an expansion of the body fluid volume is not inhibited by the antidiuretic hormone

The second point is that a similar infusion of twice isotonic Krebs solution caused a significant increase of urine volume, and naturally sodium excretion Clearly there was a marked diuresis under conditions where an increased output of antidiuretic hormone would be expected

de Wardener I am a little bit confused about these experiments in which the activity

infusion can be entirely attributed to the effect of salt loading on either the solute concentration in urine or on G F R

Croxatto I was very interested in Dr Ginsburg s observations We were able to show in cats which were under the influence of a continuous infusion of hypertonic sodium chloride solution that when they were given a rather high dose of Pitressin there was an enhancement of the diuresis which was dependent upon an increase in blood pressure

Morel We have done some experiments in rats in which we followed the changes in urine flow and the changes in sodium output by measuring radio sodium in the urine by a continuous method We injected hypertonic sodium chloride solution rapidly followed by a volume of distilled water to make the whole injection isotonic We observed an antidiuretic effect with an enormous increase in sodium concentration typical of antidiuretic hormone release When the sodium chloride was given as a single isotonic injection there was a diuretic response without an increase in the sodium concentration in the urine We have done the same experiments in animals with diabetes insipidus produced by hypothalamic lesions and they did not show this response and we think that we have stimulated the neurohypophysis by the increase in osmotic pressure

Ginsburg I was aware of and rather worried by Dr Morel s (*Bull biol France Belg Suppl* **39**, 1955) experiments as his results seemed not to agree with our own We repeated Dr Morel s experiments and when the experimental conditions were exactly the same as his we got the same results That is in rats anaesthetized with pentobarbitone rapid intravenous injection of 9 0 per cent NaCl solution followed by distilled water produced an antidiuretic response (Morel also found that ammonium chloride solutions produced greater antidiuretic effects than those following injection of NaCl solutions of the same tonicity) However in unanaesthetized rats we found that the rapid intravenous injection of 9 0 per cent NaCl followed by distilled water had a diuretic effect which was not distinguishable from that obtained after a single slow injection of the same amount of NaCl in an isotonic solution Also we found in the unanaesthetized rats that while isotonic NH_4Cl injected slowly increased the urine flow injection of strongly hypertonic NH_4Cl induced a series of violent convulsions

On the other hand when we repeated these experiments in rats anaesthetized with pentobarbitone the rapid intravenous injection of hypertonic NaCl was followed by an antidiuresis In the anaesthetized rats there were some respiratory changes following the injection The animal either stopped breathing for a few seconds or the respiration became so rapid and shallow that we could not see respiratory movements We thought it would be interesting to see what happened to the blood pressure under these conditions Firstly in unanaesthetized rats after rapid injection of hypertonic NaCl solution the blood pressure rose by about 10 mm The rats were then anaesthetized and the injection of hypertonic NaCl solution now caused a sharp fall in blood pressure of about 45 mm I would suggest then that the antidiuresis under these conditions is indeed due to antidiuretic hormone but that the hormone is released as a result of the fall in blood pressure and not by osmotic stimulation

those in Rydin & Verney's experiments on dogs or perhaps even to those in Lewis's (*Ann roy Coll Surg Engl* **13**, 36 1953) experiments in man

Chalmers Lewis Pawan and I (Chalmers, Lewis & Pawan, *J Physiol, Lond* **117**, 218, 1952) did some experiments in man in which we put venous cuffs on the legs removing from the effective circulation about 700 ml of blood by trapping it in the legs we never had any evidence of antidiuretic hormone release except when the subjects fainted

Heller That is the same result as that obtained by Professor Noble

Chalmers Yes In man short of fainting, we did not get any release of antidiuretic hormone With regard to the effect of hypertonic saline infusion in man under the conditions of the Hickey and Hare test that is 2 5 per cent sodium chloride intravenously up to a total of 700 ml in about 40 min, you usually get an antidiuretic effect although this is opposed by a sharp rise of G F R and by the osmotic diuresis

Walker Did I understand Dr Ginsburg correctly that after ether when you bleed out a rat you get an increase in the vasopressor activity in the neurohypophysis?

Ginsburg That happens in pentobarbitone anaesthesia It did not change in ether

Walker Dr Martini and I have been doing some experiments and we have found in dogs rather higher figures for antidiuretic activity in the pituitary than say Dicker & Tyler (*J Physiol, Lond* **120** 141 1953) These dogs happened to have been bled out under ether and I was hoping that that might be the explanation

Garrod When Dr Cates and I were using the Hickey and Hare test we encountered a case of primary polydipsia who showed only a minimal antidiuretic response to the infusion This patient was excreting enormous quantities of chloride at the time and we discovered later that she had been on a very high salt intake It is possible for an osmotic diuresis to mask liberation of antidiuretic hormone or the effect of Pitressin A few days later the patient responded quite normally to Pitressin

Sawyer I would just like to add something along these lines as far as the rat is concerned There the filtration rate is extremely labile and a very slight change in G F R will change the sodium load to the distal tubules tremendously Have you determined sodium or osmotic excretion during these diuresis? In a rat loaded with lots of hypertonic sodium large doses of antidiuretic hormone will give you an enhancement of diuresis and not an antidiuresis

Ginsburg I am quite sure that one of the possibilities as I have mentioned is that changes in tonicity of the urine or the G F R obscure antidiuretic effects In our experiments we were measuring urine flow at one minute intervals and the very rapid changes which occurred did not permit the use of clearance techniques In any case, we failed to observe antidiuresis following intracarotid injection of hypertonic solutions in animals in which we could show that identical salt loading did not abolish the antidiuretic effect of Pitressin although it was modified Therefore I do not think that our failure to get antidiuretic responses during the intracarotid

Polydipsia, antidiuresis and milk ejection caused by hypothalamic stimulation

by

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DURING the last half century it has become more and more obvious that the hypo thalamo neurohypophysial system plays a very important role in the regulation of water metabolism Most of the experimental work and the majority of clinical ob servations made have stressed its importance in the control of the conservation of water by the kidney but the possibility that the hypothalamus may also control water intake has been considered much more rarely Thus the polydipsia seen after an interruption of the supraoptico neurohypophysial tract or after lesions in the ventral hypothalamus has in most cases been shown to be secondary to a preceding polyuria (Richter, 1935 Fisher Magoun & Hetherington 1938 Fisher, Ingram & Ranson 1938)

Various theories have been formulated to explain the cause and the origin of the sensation of thirst The observation that fluid deprivation causes an increased serum osmotic pressure in the dog led Mayer in 1900 to suggest that thirst is in some way related to hypertonicity of the milieu interieur About twenty years later Leschke (1918) reported that the intravenous injection of strongly hypertonic NaCl solutions used as a treatment for pulmonary bleeding immediately causes intense thirst in the patient Arden (1934) observed that whereas ingestion of hypertonic NaCl or $NaHCO_3$ solutions gives rise to strong thirst ingestion of the same volume of an equally hypertonic potassium salt solution does not have this effect Similarly Gilman (1937) found that elevation of the osmotic pressure of the blood caused by intravenous injections of hypertonic solutions of NaCl causes a much greater intake of water in the dog than an equivalent rise obtained by the injection of urea Since Cannon s (1918) dry mouth theory has been more or less disproved (Montgomery 1931 Bellows & van Wagenen 1939 Steggerda 1939 Archdeacon, Presnell & Walton 1949 and others) these and other observations have made it very likely that the main factor responsible for the elicitation of thirst is a relative dehydration that is a deficit of water relative to certain extracellular electrolytes It has however remained open to discussion as to how and where in the body a relative dehydration becomes manifest as an urge to drink The sensation of thirst has thus been thought either to be of general origin or to originate from special thirst receptors localized somewhere in the body

Since the classical studies of Verney (1947) it has been generally accepted that osmoreceptors localized in the hypothalamus control the conservation of water by the kidney by regulating the release of antidiuretic hormone from the neurohypo

Martini In connexion with what Dr Ginsburg said about the relationships between buffer nerves and antidiuretic hormone secretion, I want to emphasize that we have observed that in dogs after bilateral vagatomy and section of both carotid sinus nerves, the amount of antidiuretic activity in the cerebospinal fluid is increased This augmentation of antidiuretic hormone secretion maybe of some importance in maintaining the rise of blood pressure generally observed in debuffered dogs

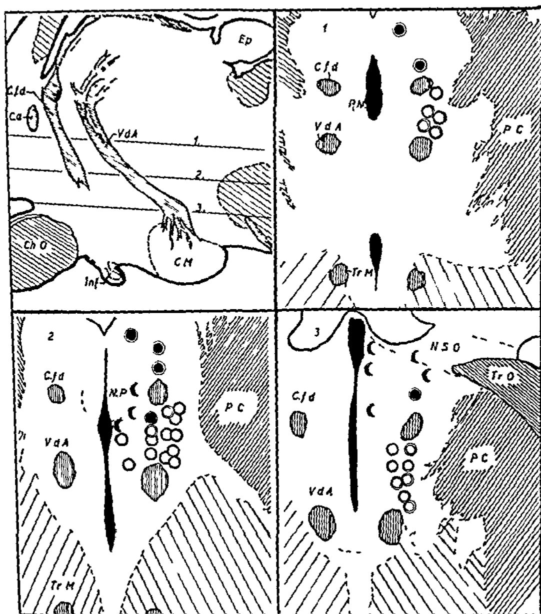


Figure 1

Points in the hypothalamus where electrical stimulation evoked drinking, inhibition of water diuresis and milk ejection
Diagrams of a sagittal section and corresponding to the lines labelled 1 2 and 3 three horizontal sections (1 2 and 3) through the hypothalamus of the goat

Black circles Points where electrical stimulation caused drinking inhibition of water diuresis and milk ejection
Open circles Points where drinking was obtained in the absence of antidiuresis and milk ejection
Black crescents Points where stimulation produced inhibition of water diuresis and milk ejection but no drinking

Ca	Commissura anterior	*NP*	Nucleus paraventricularis
Cfd	Columna fornicis descendens	*NSO*	Nucleus supraopticus
CM	Corpus mammillare	*PC*	Pedunculus cerebri
Ch O	Chiasma opticum	*Tr M*	Tractus Meynert
Ep	Epiphysis	*VdA*	Tractus Vicq d Azyr
Inf	Infundibulum		

(From Andersson & McCann 1955*b*)

physıs Wolf (1950), on the basıs of an osmometrıc analysıs of thırst ın man has suggested that the sensatıon of thırst orıgınates ın the same or sımılar osmoreceptors as those which Verney had found to regulate the release of antıdıuretıc hormone from the neurohypophysıs Earlier than thıs, however, evidence had been gıven that the hypothalamus was ın some way ınvolved ın the regulatıon not only of water excretıon but also of water ıntake Baıly & Bremer (1921) had found that lesıons ın the hypo thalamus of dogs sometımes produced polydıpsıa on the day of operatıon, although polyurıa dıd not appear untıl the next day Also a few clınıcal cases have been re ported ın which polydıpsıa dıd not seem to be the consequence of a precedıng polyurıa (Fulton & Baıly 1929 Kourılsky, 1950 and others) Some of Hess s (1932) experı ments also ımplıed that the urge to drınk may orıgınate from the hypothalamus Thus Brugger (1943) revıewıng these experıments ın which electrıcal stımulatıon of the hypothalamus ın cats had caused abnormal hunger noted that ın some ın stances the anımals had shown a preference for mılk as contrasted to meat which suggests that they mıght also have drunk water ıf ıt had been offered to them

POLYDIPSIA ELICITED BY INTRAHYPOTHALAMIC INJECTIONS OF HYPERTONIC SALINE

That hypothalamıc stımulatıon can cause polydıpsıa was ultımately shown ın the goat (Andersson, 1952, 1953) Andersson (1951) had found that ıntracarotıd ınjec tıons of hypertonıc salıne could cause mılk ejectıon ın the lactatıng goat Thıs ob servatıon led to a study of the effect of ıntrahypothalamıc ınjectıons of 0 1 to 0 2 ml of 1 5 to 2 per cent NaCl solutıons on water ıntake and ıt was found that ınjectıons made medıally ınto a faırly wıde area ın the hypothalamus often caused drınkıng of large amounts of water Thıs polydıpsıa was followed by a polyurıa which, however, dıd not commence untıl about two hours after the ıngestıon of water Later, Anders son & McCann (1955*a*) obtaıned the same effect from ınjectıons of much smaller amounts of hypertonıc salıne (less than 0 01 ml of a 2 or 3 per cent NaCl solutıon) gıven ın approxımately the same regıon of the hypothalamus The maın dısadvantage of these ınjectıons was that the effect could not be easıly repeated The same authors (1955*a*, *b*) therefore trıed electrıcal stımulatıon usıng Hess s (1932 1949) technıque

POLYDIPSIA INDUCED BY ELECTRICAL STIMULATION

In general the results of electrıcal stımulatıon showed that thıs was a convenıent and effective way to study hypothalamıc polydıpsıa ın the goat experımentally ınduced drınkıng could be repeated at wıll and the area concerned ın the response could be more closely localızed than ın the ınjectıon experıments The area was found to extend from the dorsal ınto the ventral hypothalamus, to have ıts anterıor pole approxımately at the level of a transverse sectıon through the anterıor commıssure and to reach posterıorly as far as to the tract of Vıcq d Azyr (Fıg 1 black and open cırcles) The latency perıod before the onset of drınkıng was short—ın many cases only about 5 sec —and the anımals stopped drınkıng a few seconds after the end of stımulatıon As a rule they drank contınuously durıng the perıods of stımulatıon and, by prolonged stımulatıon could be ınduced to drınk volumes of water up to 40 per cent of theır body weıght wıthın short perıods of tıme, an overhydratıon which was seen to produce haemolysıs haemoglobınurıa and other sıgns of water ıntoxıcatıon



Plate 1

The appearance cf a goat mmediately after it had drunk 15 l f wate following electrical stimulation of the d nking area n the hypothalamus (*A*)
B shows the same goat the follow ng morning wh n most cf the water had been elim nated

(Plate 1). When tested during such periods of stimulation the animals would accept a weak mixture of urine and water but would refuse to drink a more concentrated solution. Also, in other ways, the character of the polydipsia seen in these experiments gave the impression that the stimulus did cause a conscious urge to drink. Thus the animals went to the water by themselves even when it was placed in the opposite corner of the pen.

From these observations, taken together with the remarkable reproducibility of the polydipsic effect, the question arose whether the goats could get conditioned to the hypothalamic stimuli. Andersson & Larsson (1956) therefore made attempts to condition the polydipsic effect of electrical stimulation in the hypothalamus of the goat. In spite of up to 110 combinations of neutral and electrical stimuli, the neutral stimuli alone never caused the animals to drink—they did not even show any interest in the water offered. However, electrical stimulation of the 'drinking centre' combined with neutral stimuli never failed to evoke drinking of water after a very short latency period.

INHIBITION OF WATER DIURESIS AND MILK EJECTION AS RESULTS OF HYPOTHALAMIC STIMULATION

In most of the experiments done by Andersson & McCann (1955*b*) to 'map' the localization of the 'drinking area' of the hypothalamus, the effects of stimulation on water diuresis and on the flow of milk from cannulated teats were also measured. Stimulation in the anterior part of the 'drinking area' was found to cause inhibition of water diuresis of neurohypophysial type and ejection of milk simultaneously with polydipsia (Fig. 1, black circles). Stimulation in its posterior part did not seem to cause any release of hormone from the neurohypophysis (Fig. 1, open circles). Stimulation within or in regions adjacent to the paraventricular nuclei did not cause drinking of water, but did produce inhibition of water diuresis and ejection of milk (Fig. 1, horizonal section 2, black crescents). Similarly stimulation within the supraoptic nuclei, or in the vicinity of the supraoptico-neurohypophysial tract, caused, as expected, inhibition of water diuresis (Harris, 1947) and milk ejection (Andersson, 1951; Cross & Harris, 1951), but failed to produce polydipsia (Fig. 1, horizontal section 3, black crescents). The inhibition of water diuresis seen in these experiments lasted from 45 to 60 min. During the period of inhibition there was a marked rise in the urinary concentration of Na^+, K^+ and Cl^- and in specific gravity; the total amounts of electrolytes excreted showed less consistent changes. Usually more Na^+ and Cl^- was excreted during the period of inhibition, but sometimes there was no obvious increase or even a small decrease. Increases in the excretion of potassium were even less consistent. There was no significant change in endogenous creatinine clearance during the period of inhibition.

Milk ejection, which was always combined with antidiuresis, began one to two minutes after the onset of stimulation, but since it was only visually determined by the increased flow of milk from the cannulated teats, no quantitative measure of the response could be obtained from the experiments.

DISCUSSION

The experiments described above indicate that the hypothalamus plays an important role in the regulation of water intake, but they do not show whether the hypothalamic drinking area is really essential for the development of an urge to drink. A number of clinical cases have been reported in whom injury to the basal portion of the brain was followed by increased plasma sodium and chloride concentrations. This finding has been interpreted by some workers as the result of damage of either a thirst centre (Enstrom & Liebman 1953, Elliot 1955, Rothballer & Dugger, 1955) or a centre regulating the electrolyte metabolism (Welt, Seldin, Nelson, German & Peeters 1952, Cooper, 1953). Further Stevenson (1949) and Stevenson, Welt & Orloff (1950) have reported a decreased ratio of water to food intake, a decreased response to water loads and moderately elevated plasma sodium concentrations in rats with hypothalamic obesity. These findings were interpreted as indicating the existence of a state of hypothalamic hypodypsia. Still more striking are results briefly reported by Witt, Keller, Batsel & Lynch (1952), who found that the extension of the lesions of diabetes insipidus dogs dorsally and laterally may result in temporary or even permanent adipsia. Their adipsic dogs ate and took tasty fluids like milk and broth but refused to drink water even when they became severely dehydrated. Similar experiments were later done by Andersson & McCann (1956). Having determined the localization of the drinking area in the hypothalamus of the goat, they tried to inactivate as much as possible of the corresponding part of the brain in dogs by electrocoagulation without destroying the supraoptic neurohypophysial connexions. Such lesions caused permanent hypodipsia or adipsia lasting up to 14 days after the operation; the animals, in contrast to the absence of thirst, ate and drank milk and broth readily when offered. The degree of hypodipsia seemed to be roughly proportional to the extent of destruction of the same area of the hypothalamus which on stimulation evokes drinking in the goat. However, no correlation was found in these experiments between the adipsia and the eventual presence of diabetes insipidus.

Thus strong evidence is now available that an essential thirst centre is located in the hypothalamus, since stimulation within a well defined hypothalamic region produces drinking, whereas destructive lesions in the same region result in a marked decrease in water intake.

The thirst receptors seem to be localized in the portion of the hypothalamus outlined by the response to electrical stimulation but the physiological stimulus has not been determined. The osmotic stimuli discussed on p. 132 may have activated thirst receptors in the hypothalamus but it cannot be ruled out that the polydipsia seen after intrahypothalamic injections of hypertonic saline was due to a non specific effect. Further it is not clear from our results whether the regulation of water intake and that of water excretion involves distinct groups of receptors. If all the presumed osmoreceptors in the hypothalamus have a dual function in the regulation of both water intake and output, one would expect that their stimulation would induce both drinking and a release of antidiuretic hormone from the neurohypophysis. Both drinking and inhibition of water diuresis were indeed found after stimulation of the rostral portion of the drinking area. Thus if the osmoreceptors have a dual function they

Electrical stimulation of the paraventricular nuclei or adjacent sites caused inhibition of water diuresis and milk ejection, but no drinking of water

The significance of these results is discussed

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would be expected to lie in that region In this case the caudal portion of the 'drinking area' from which antidiuresis could not be elicited might represent fibres passing from the receptors to higher centres or may contain 'thirst receptors' of another kind A second explanation would be that there are two groups of osmoreceptors in the hypothalamus, i e one group concerned with the conservation of water by the kidneys and the other with water intake The two groups would then be localized in two areas which overlap in the region where both drinking and inhibition of water diuresis can be evoked by stimulation Thirdly since stimulations in or adjacent to, the paraventricular nuclei caused inhibition of water diuresis, the inhibition of water diuresis, which was obtained in the region where drinking could also be induced may have been due to stimulation of fibres running from the paraventricular to the supraoptic nuclei and/or to the neurohypophysis The results presented do not allow a decision between these and other explanations

The fact that antidiuresis could be obtained when stimuli were applied within the paraventricular nuclei or in their vicinity, is at variance with Harris s (1947) findings in the rabbit that such stimuli do not cause inhibition of water diuresis

Milk ejection was always seen combined with inhibition of water diuresis This is consistent with the hypothesis (Abel & Nagayama 1920) that only one hormone is released from the neurohypophysis or that both antidiuretic and oxytocic hormones are released together The observation (Andersson, 1951) that intracarotid injections of hypertonic saline can cause milk ejection in the goat and the more thorough studies of Abrahams & Pickford (1954) showing that a variety of stimuli (such as emotion intracarotid or intravenous injection of hypertonic saline solutions or of acetylcholine) inhibit water diuresis by liberation of antidiuretic hormone and also cause increased uterine activity in the dog may also be interpreted as supporting the unitary hypothesis However although milk ejection could easily be demonstrated by observing milk flow in the goat no conclusions could be drawn as to the amount of oxytocic activity responsible for this effect

Milk ejection caused by electrical stimulation in the paraventricular region, has also been described by Cross (1955) in the rabbit but only after adrenalectomy Cross (1953) showed in the same species that adrenaline blocked the response of the mammary gland to oxytocin by a peripheral action He found that stimulation near the paraventricular nuclei also activates the sympathetico adrenal system, and that this in turn inhibits the response of the mammary gland to oxytocin It may be that stimulation of the paraventricular nuclei in the larger brain of the goat does not activate the sympathetico adrenal system sufficiently to cause peripheral blocking of the mammary response

SUMMARY

Injections of hypertonic sodium chloride solutions into and electrical stimulation within a fairly wide region of the hypothalamus, were found to induce polydipsia in the goat Electrical stimulation of the anterior portion of this 'drinking area caused inhibition of water diuresis of the neurohypophysial type and also milk ejection simultaneously with the drinking of water Antidiuresis and milk ejection were not observed when the posterior part of the drinking area was stimulated

Electrical stimulation of the paraventricular nuclei or adjacent sites caused inhibition of water diuresis and milk ejection, but no drinking of water

The significance of these results is discussed

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Discussion

Chairman G W Harris

Folley Dr Andersson spoke of adipsia and referred to dogs which would not drink water He also said that these dogs drank milk and since milk contains some 87 per cent water is it correct to speak of these animals as being adipsic? I should also like to ask Dr Andersson if he used the horns of the goats to support his electrodes

Andersson It is not necessary to use goats with horns We find, however, that the horns protect the electrodes

The dogs referred to by Dr Folley were hyperphagic that is they ate ravenously and we interpreted the intake of milk as eating rather than drinking We reduced some dogs to a severe state of dehydration but they would still not take water although they would take milk

Harris, G W Could Dr Andersson relate the region he stimulated to produce polydipsia with that reported by the Yale group (Delgardo & Anand *Amer J Physiol* **172**, 162, 1953) which resulted in change of feeding habits If I remember correctly they stimulated a little more laterally

Andersson Yes, a little more laterally and more posteriorly It is possible to see the combination of both effects in that there are points which give simultaneous drinking and eating responses However, drinking and eating responses can be obtained individually at other points, showing that the areas concerned are at least partly separated

Sawyer I want to report an interesting phenomenon noticed originally in my laboratory some years ago by Gordon Gilbert who has since pursued this work in the Department of Anatomy at New York University He found that centrally located lesions in the region of the posterior commissure in the rat that is in an area quite distinct from that described by Andersson in the goat, cause complete adipsia Lesions which overlapped the sub commissural organ, whatever that may be cause the rats to cease drinking water and, if allowed, to dehydrate themselves to death They would, however take milk if offered and this could provide the necessary fluid On withdrawal of the milk, however, they would again refuse water

Harris, G W This is a most interesting observation I believe the sub commissural organ has a neurosecretory function Perhaps Professor Bargmann would comment on this

Bargmann There is a considerable amount of Gomori positive substance in this area but it is not necessarily the same as that found in the neurohypophysis I have no information whether its secretion or release is evoked by various stimuli

Cross I should like to record my admiration of the excellent stimulation experiments of Dr Andersson He has extended the use of the Hess technique to unanaesthetized sheep and goats and has obtained new information about special aspects of ruminant physiology, in addition to contributing to our knowledge of the physiology of the neurohypophysis

With regard to the induction of drinking by hypothalamic stimulation I would like to ask Dr Andersson if he feels confident that the goats really feel thirsty or whether the response might result from activation of motor mechanisms In his film it appeared that the latency of the drinking response grew less with repetition of the stimuli until it became so short as to make it seem unlikely that the animal had time to experience thirst subjectively before starting to drink again

Dr Andersson s experiments appear to be the first demonstration that stimulation of the paraventricular nuclei may cause release of antidiuretic hormone It seems clear moreover from his results that such stimulation also releases oxytocin as shown by milk ejection Some of my own experiments agree with the latter finding Exploratory stimulation of the hypothalamus in a series of rabbits has so far yielded twelve animals in which histological reconstruction of the stimulation points indicates that localized stimulation of the paraventricular nucleus induces release of oxytocin as shown by either ejection of milk or by the contractile response of the oestrogenized uterus *in situ*

It might throw some light on the problem of what is released from the neurohypophysis and on the role of the paraventricular and supraoptic nuclei in determining this if simultaneous recordings were made of antidiuretic and milk ejection

(or uterine) responses following localized stimulation of these nuclei, and the effects compared with *injections of* vasopressin and oxytocin

Andersson It is of course impossible with certainty, to interpret whether the goats experience a sensation of thirst However, we think it likely since in most cases they actively search for water

There is a facilitation of response on repetition seen as a decrease in the latency period It has been suggested to us that this may be a conditioned reflex by which the animal learns to respond to the light signal coincident with switching on the electrical stimulus We have investigated this experimentally and have found that the animals do not become conditioned in this way

Dale There is insufficient time I take it for the mouth to be dry

Andersson That is so and one can prolong the drinking even though the mouth is being bathed with water

Dale There is no effect on the salivary secretion?

Andersson I do not think that could show so quickly

Dale No

de Wardener It might be possible to investigate the difference between milk and water by applying cocaine to the tongue and nose

Jewell It seems likely that these dogs were attracted to milk as a food and not as a drink since the dogs which Dr Andersson and I overhydrated would show a distinct aversion to water but would accept milk

Garrod Dr Andersson s elegant experiments have shown that polydipsia can be obtained by stimulating the appropriate hypothalamic centres Is there any evidence that similar effects may result from *lesions* to the hypothalamus in goats or any other species This is an important problem in clinical medicine Kourilsky (*Rev neurol* **74**, 264, 1942) has described a case of primary polydipsia in which there was a hypo thalamic lesion without a coincident neurohypophysial defect

Andersson I think this was an almost isolated observation of a chronic condition It was a cyst pressing on the hypothalamus otherwise there are only a few observations of an acute nature

Noble Dr Andersson s experiments evidently show that in some circumstances one can lead an animal to water and make him drink! Do you have any information as to whether the anterior pituitary is essential for the maintenance of drinking follow ing stimulation as seen in the goats in your film? Has the hypophysectomized goat been studied?

Cowie I have hypophysectomized a few goats and the animal attendants have re ported a great increase in water consumption for a few days after the operation So far, however, no measurements of water intake have been made

Conditions under which posterior pituitary hormones increase sodium and potassium excretion by the kidney

by

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A NUMBER of workers have noticed that whatever the stimulus they applied to the posterior pituitary the gland liberated simultaneously both the oxytocic and vaso pressor factors (Harris 1955) The methods of stimulation and the species of animal used have varied, so have the proportional amounts of the two substances but in every instance more oxytocin than vasopressin appears to be released Oxytocin is of further interest in that it has been assigned a role only in one sex in mammals a role which may never be played or at best only intermittently A number of attempts have been made to determine whether oxytocin has a more general activity than that on the uterus and mammae for instance in the control of kidney function In their investigations most workers have used oxytocin in doses comparable with the anti diuretic dose of vasopressin and have obtained negative results However Dicker & Heller (1946) reported that when given subcutaneously to rats in doses of 3 mU /100 g of rat glomerular filtration rate (GFR) and renal plasma flow (RPF) were in creased and Cl reabsorption diminished Taking recent work into account it seemed desirable to re examine the effect of vasopressin and oxytocin on the kidney using larger doses of oxytocin than previously and also since the two factors are always liberated together to test whether when given simultaneously they show mutual enhancement or interference The observations to be described then had as their object a fresh study of the effect of vasopressin and oxytocin on the volume of urine excreted on its Na and K content and on the clearance rates of diodone and creati nine and also the discovery if possible of the natural conditions in which the two substances are released to produce any effect noted

METHODS

The observations were made on conscious dogs both during water diuresis and at resting rates of urine flow Vasopressin and oxytocin were given either as single in travenous injections or as intravenous infusions at a rate of 6–7 ml solution/hr A number of experiments were done using Pitocin and Pitressin (Parke Davis and Co) and a number using highly purified vasopressin (both the arginine and lysine varieties, though almost entirely the former) and highly purified oxytocin The purified sub stances were generously supplied by Dr du Vigneaud The doses of oxytocin used

were chosen on the basis of information obtained by Abrahams & Pickford (1954) who found that the intracarotid injection into a 12 kg dog of a small volume of hypertonic NaCl solution induced antidiuresis equivalent to that following the intravenous injection of 3 mU Pitressin and, at the same time, an increase in spontaneous uterine activity equivalent to that following the injection of 80–100 mU Pitocin, i e , apparently 25–30 times as much oxytocin as vasopressin was released as a result of an osmotic stimulus In the following experiments, then, vasopressin was used in antidiuretic doses, namely for a single intravenous injection 0 2 to 0 4 mU /kg body weight, and for infusion at the rate of 0 01–0 02 mU /kg /min oxytocin was given in doses of 2 5 to 15 mU /kg body weight for single injections or infused intravenously at the rate of 0 1 to 0 2 mU /kg /min Clearances of creatinine and diodone were measured by the method of Pickford & Ritchie (1945) or by the technique of a priming injection followed by a maintaining infusion Again the volume infused was at the rate of 6 to 7 ml /hr Na and K analyses were made by means of an EEL flame photometer The dead space of the urine collecting system was reduced as much as possible and in a number of instances the external part of the uretheral catheter was emptied at the end of each urine collection period by blowing air through it from a T piece inserted at the site of emergence of the catheter from the vagina As far as possible, in all graphs correction has been made for dead space of the urinary system

RESULTS

The earliest experiments compared the effects of single injections of Pitocin and Pitressin On some occasions it was found that Pitocin greatly increased Na excretion during water diuresis whereas Pitressin did not have this effect (Fig 1), (Brooks & Pickford, 1956) When the purified oxytocin became available it was seen that this substance never increased Na excretion during water diuresis (Fig 2) It became necessary, then to determine whether synergism between Pitocin and its vasopressor contaminant was responsible for the positive effect seen or whether either Pitocin or Pitressin contained some other active factor Both preparations therefore were tested after treatment with thioglycollic acid, NaOH ascorbic acid and trypsin All these agents destroyed any activity previously present and did not unmask one hitherto concealed Thus the enhanced excretion of Na sometimes seen during water diuresis after an intravenous injection of Pitocin was difficult to explain unless some samples of Pitocin contain a greater than usual amount of vasopressin and there is a synergism of the two active substances

A careful study of the effect of single injections of vasopressin and oxytocin (Fig 2) on Na excretion during water diuresis suggests that although these substances do not strikingly increase the excretion of Na or K they do prevent the steady fall in output/min which is the rule in simple water diuresis (Fig 5) On the other hand when oxytocin is injected at resting rates of urine excretion it has a marked effect on Na excretion and a definite one on K excretion (Fig 3) In similar circumstances vasopressin only slightly increased the rate of Na excretion but did in the instance shown in the graph clearly increase the excretion of K The positive action on K excretion was not always seen The difference in the effects of oxytocin and vasopressin does not depend on the dose given As others have shown, small doses of oxytocin have

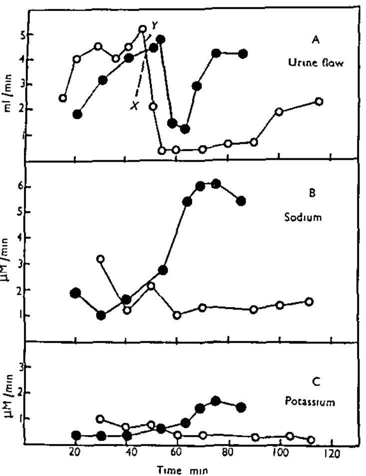


Figure 1

The effect of Pitocin and Pitressin on urine flo\ and \a and K excretion during \ater diures s (Thisbe 15 kg) 300 ml water by mouth at zero time Curves ●——● intravenous injection of 100 mU Pitocin at λ Curves ○——○ intravenous injection of 4 mU Pitressin at Y

Figure 2

The effect of oxytocin and vasopressin on urine flo\ and \a and K excretion during water diuresis (Thisbe 15 kg) 300 ml water by mouth at zero time Curves ●——● intravenous injection of 60 mU oxytocin at X Curves ○——○ intravenous injection of 4 mU vasopressin at Y

Fi₅ure 3

The effect of oxyt c n and va opress n on u n flow and Na and K excretion at a low rate of urin fl w (C) lop 8 5 kg) C rv ●——● 50 mU oxytocin ntra en u ly at X Cu O——O U a op n t av n ou ly at Y

Figure 4

Th ff t of an nfu on of a oj on u ne flo v and Na a d K ex t o du ng ι at d u (C) lop 8 5 kg) oo ml at by mo tl at z o m V a oj fu d at tl t of o U/m n lu g p od d a d

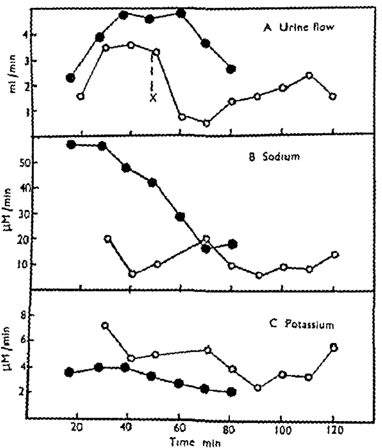


Figure 5

The effect of water diures and of oxytocin plus vasopress n i jected during diuresis on rate of urine flo v and Na and K excretion (Cyclops B 5 kg) 200 ml water by n outh at zero time Curves ●— ● contr l v ater d ure is Curves O——O 50 mU oxytocin plus 1 6 n U vasopre sin injected intravenou ly at X

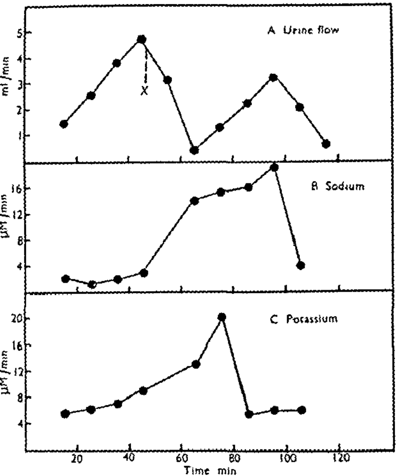


Figure 6

The effect of oxytocin plus I itressin on rate of urine fl w and on Na and K excre t on during v ater diure s (Jes 21 kg) 3.. ml v ater l y mouth at zero time 1.0 mU oxytocin plus 3 mU I itressin injected intravenou ly at X

Figure 3

The eff ct of xyt cin and vasop n on u in flo and Na and K ex ret on at a lov rate of u ne flow (C) lop 8 5 kg) Curves ●——● 50 n U oxytocin int venou ly at X Cu O——O 2 U asop ss n t av n ou ly at Y

Figure 4

Tl ff t of an nfu of a ssnonu e flo v an l Na and K x ret o lu g wat l u (C) l 8 5 kg) 200 n l wa by o l at o ne Va ss nt d at tl at of o n U / n du ng pe od i l a d

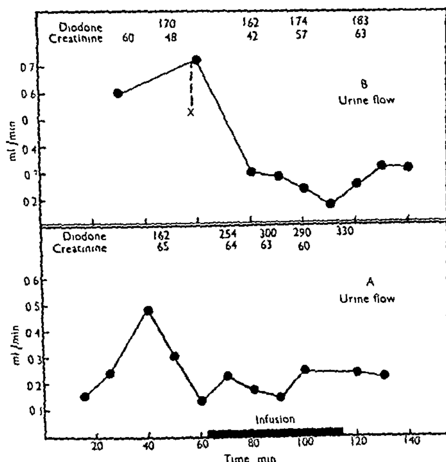


Figure 9

The eff ct of Pitocin and of Pitocin plus a opre.sin on clearances of d odone and creatinine at a low rate of urine flow *A* (Thisbe 15 kg) P tocin infused ntravenously during tin e indicated at rate of mU /min Related clearance values abo e in ml /min *B* (Jess 1 kg) 75 mU P tocin plu 2 mU vasopressin inject d intra enously at *X* Related clearance values above n ml /min

no discoverable action on renal activity 40 or 20 mU vasopressin only transitorily i e for one or at most two urine samples increased the excretion of both Na and K This action of vasopressin could not be differentiated from the immediate effect of strongly antidiuretic doses of adrenaline or 5 hydroxytryptamine There is then, a clear difference between the action of single injections of vasopressin or oxytocin on Na excretion, and between the effects of oxytocin at fast and slow rates of urine flow However when vasopressin was infused during water diuresis the results differed from those of a single injection As can be seen in Fig 4 it did then clearly increa e the excretion of both Na and K These results are in agreement with those of Anslow and Wesson (1955) It seems that vasopressin needs more time to produce its effect on Na than does oxytocin

The next step was to see whether oxytocin and vasopre sin given simultaneously

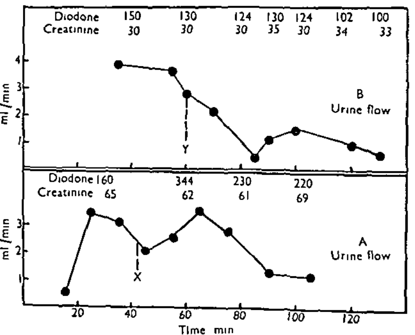


Figure 7

The effect of oxytocin plus vasopressin on urine flow and Na and K excretion at a low rate of urine flow (Cyclops 8 5 kg) 50 mU oxytoc n plu 2 5 mU va opress n injected intravenou ly at X

Figure 8

The effect of oxytocin and of oxytocin plus va opres in on renal clearance of diodone and creatinine during water diure is *A* (Thisbe 15 kg) 300 ml water by mouth at zero time 75 mU oxytocin injected intravenously at X Related clearance values above in ml /min *B* Cy lops 8 5 kg 200 ml water by mouth at z ro tim 25 mU oxytoc n plu 2 mU vasop e sin inj ct d nt av no ly at *Y* Relat d l a a ce valu abo e in nl / n n

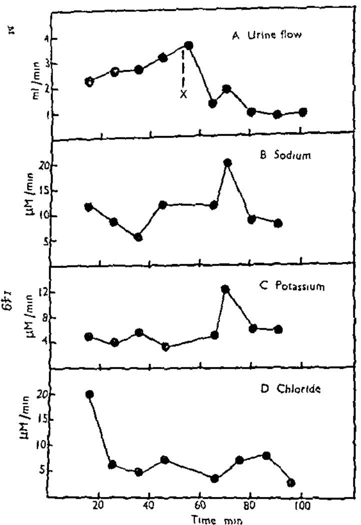


Figure 10

The effect of 0·89 M sucrose solution on urine flow and Na, K and Cl excretion during water diuresis (Cyclop, 8·5 kg), 200 ml water by mouth at zero time, 10 ml sucrose solution injected intravenously at X

Figure 11

The effect of 0·89 M sucrose solution on urine flow and Na and K excretion at a low rate of urine flow (Thisbe, 15 kg), 10 ml sucrose solution injected intravenously at X

had an action different from that of either given alone The effect of the two given together in a single injection during water diuresis varied, at times the increased electrolyte excretion was minimal, but the normal fall in rate of excretion was prevented (Fig 5), at other times there was an increase in rate of excretion of Na and K never seen during diuresis following the injection of either oxytocin or vasopressin alone (Fig 6) At a low rate of urine flow the two factors injected together maintained a high level of Na excretion not seen in their absence nor following vasopressin alone (Fig 7) but not as great as that following oxytocin alone

Measurements of the clearances of diodone and creatinine brought out another difference between the actions of oxytocin and vasopressin and also showed the existence of an antagonism Previous workers have found that the vasopressor fraction of posterior pituitary extracts given in antidiuretic doses does not alter either RPF or GFR (Maxwell & Breed, quoted by Smith 1951 Sellwood & Verney, 1955) In Figs 8*a* and 9*a* is shown the invariable effect of oxytocin on renal clearances in dogs regardless of whether it was injected during water diuresis or at low rates of urine flow The clearance of creatinine was unaffected whilst that of diodone was increased by 75–100 per cent whether the oxytocin was given as a single intravenous injection or as an infusion At fairly high rates of urine flow the errors in clearance calculation due to renal dead space are small compared with those during low rates of urine flow Nevertheless the magnitude of the increase in diodone clearance is such that it cannot be discounted by any possible error due to dead space Further the increase begins too early to be easily explained by a process of cell storage of diodone In the example given and in other instances too there was always about the same proportionate increase in diodone clearance Here then was a second difference between the effects of oxytocin and vasopressin on the kidney The antagonism between them was seen when the two substances were injected simultaneously During water diuresis 2 mU vasopressin entirely suppressed the increased diodone clearance which would be expected to follow the intravenous injection of 25 mU oxytocin alone (Fig 8*b*) Fig 9*b* shows that at a low rate of urine flow 2 mU vasopressin cancelled the expected action of 75 mU Pitocin on diodone clearance It follows from this then, that the observed fact that during antidiuresis of pituitary origin the clearances remain normal does not controvert the possibility that oxytocin is at the same time released in amounts equivalent to 75–100 mU This means that the liberation of such large amounts of oxytocin may well be a fact and not merely apparent

This analytic aspect of the problem obviously still needs more work, but it was of interest to pass to the next stage and see if stimuli presumed to activate the posterior pituitary induced urinary effects which could be explained by the information so far obtained The stimulus chosen was an osmotic one viz the injection of hypertonic sucrose solution in the 0 89 M concentration used successfully by Verney (1947) The intravenous route of administration was used because until the present time no dog with a carotid loop has been available The intravenous route is not wholly satisfactory since to induce antidiuresis moderately large volumes of sucrose solution have to be injected and may well have some peripheral action in addition to that on the hypothalamus The observations must be repeated using small volumes of sucrose solution injected into the carotid artery Figure 10 shows the effect of injecting

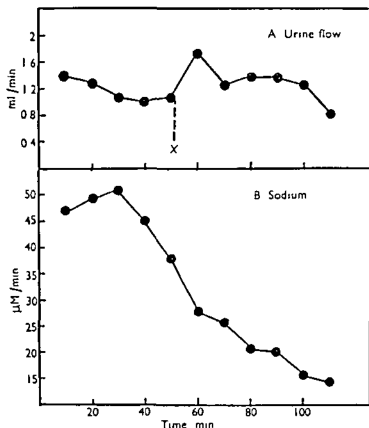


Figure 14

The effect of 0 89 M sucrose solution on urine flow and Na and K excretion in a dog with diabetes insipidus (Meg 20 kg) 20 ml sucrose solution injected intravenously at X The amount of K excreted was constant and too small to be worth recording

hypertonic sucrose solution during the course of a water diuresis There was a passing increase in Na and K excretion following the injection Figure 10 also shows that Cl excretion was unaffected Figure 11 shows an example of what occurred at low rates of urine flow Not in all instances was the increased excretion of Na and K as marked as in this particular case, but it was always definite The events following the intravenous injection of hypertonic sucrose solution then are not unlike those which may be seen following the simultaneous injection of oxytocin and vasopressin (Figs 5 and 7)

There is yet another indication that one or both posterior lobe hormones are important in the excretion of Na In a dog with diabetes insipidus following section of the supraoptico hypophysial tracts the injection of Pitocin at high or low rates of urine flow induced normal effects thus during water diuresis no increase in Na excretion followed the injection (Fig 12) but at resting rate of urine flow both Na and K excretion increased (Fig 13) However the intravenous injection of hypertonic

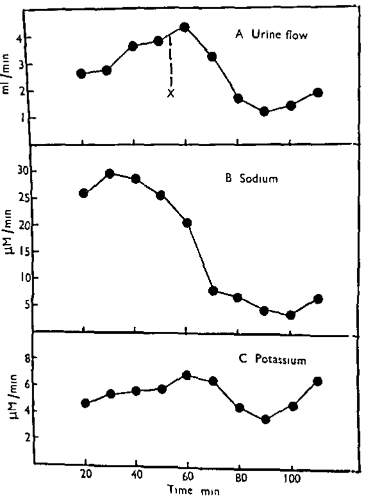


Figure 12

Effect of Pitocin on urine flo and Na and K xcret on dur ng wate diuresis in a d g with d abetes ins p dus (M g 20 kg) 300 ml water by moutl at zero tim 10 mU P tocin injected intravenously at X

Figure 13

The effect of Pitoc n on u ine flo v an l Na and K exc et on at a lo ate of urin flo in a dog with d abet n p du (M g 20 kg) 150 mU P toc n i j te l nt a e u l at X

when the rate of urine flow was low had effects on Na and K excretion not unlike those due to the administration of mixtures of oxytocin and vasopressin

7 A dog with diabetes insipidus responded normally to intravenous injections of oxytocin, but showed no increase in Na excretion following intravenously administered 0 89 M sucrose solution, either at high or low rates of urine flow

We wish to express our deep gratitude to Dr du Vigneaud for gifts of highly purified oxytocin and vasopressin

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Discussion

Chairman G W Harris

Sawyer I think it is an extraordinarily interesting suggestion that oxytocin may have a physiological role in the regulation of renal function Demunbrun *et al* (Demunbrun Keller Levkoff & Purser *Amer J Physiol* **179** 429 1954) reported that large doses of oxytocin restored depressed renal functions in dogs with surgically induced diabetes insipidus They have not reported however on the use of synthetic oxytocin This is important if we are to rule out the possibility that the effects they observed were due to contamination with anterior lobe substances since their dogs showed some evidence of mild adenohypophysial insufficiency

Your hypertonic sucrose injections gave increased sodium excretion at low urine flow Are you sure this is not the result of an osmotic diuresis? It would be interesting to see if the equivalent experiment could be done with equi osmotic concentrations of urea to promote osmotic diuresis without stimulating liberation of antidiuretic hormone

sucrose solution, which, had the dog been normal would have increased Na excretion was wholly without this effect (Fig. 14).

The above results do not give an answer to the question whether the action of oxytocin in the kidney is a physiological one. Also, these observations tell us little of the mode of action of the hormones on Na excretion, nor why K is sometimes definitely affected in the same sense as Na and at other times not at all or in an opposite direction. But the observations on clearances do give one piece of positive information: that an increase in Na excretion following oxytocin administration is not directly related to a change in plasma flow as measured by diodone clearance. The negative response to hypertonic sucrose solution of the dog with diabetes insipidus adds to the evidence that posterior lobe hormones must play some part in control of electrolyte excretion. There is no evidence that oxytocin is ever liberated almost alone in amounts sufficient to increase Na excretion; on the contrary, such evidence as has been obtained in these experiments suggests that the hormones act in concert, oxytocin being perhaps the more rapidly acting as far as Na excretion is concerned, and vasopressin far the more powerful for water retention. If oxytocin and vasopressin combine together to rid the body of Na and at the same time retain water, this activity would be useful at certain stages of dehydration.

Until more is known about the proportional amounts of oxytocin and vasopressin released by a single species in response to different types of stimuli further discussion can be no more than speculation.

SUMMARY

1. Observations were made on dogs of the effect of the oxytocic and vasopressor factors of the posterior pituitary on the rate of urine flow, on the excretion of sodium and potassium and on renal clearances of diodone and creatinine both during water diuresis and at low rates of urine flow. Vasopressin was used in antidiuretic doses and oxytocin in concentrations 20 to 30 times greater.

2. Highly purified oxytocin given as a single intravenous injection or as an intravenous infusion during water diuresis had little or no effect on the excretion of Na or K. If administered when the rate of urine flow was low oxytocin greatly increased Na excretion, and sometimes also that of K.

3. Highly purified vasopressin given as a single intravenous injection during water diuresis or when the rate of urine flow was low, did not strikingly increase the excretion rate of Na. Occasionally the excretion rate of K was increased. Given as an intravenous infusion during water diuresis vasopressin increased the excretion rate of both Na and K.

4. If the purified substances were injected together during diuresis or when the rate of urine flow was low the result was a sustained and moderate, or sometimes a marked, increase in excretion rates of both Na and K.

5. Measurements of diodone and creatinine clearances showed that at high or low rates of urine flow the administration of oxytocin was followed by a period when the diodone clearance increased by 75–100 per cent, though creatinine clearance was unaffected. When oxytocin and vasopressin were administered simultaneously there was no subsequent change in either diodone or creatinine clearance.

6. The intravenous injection of 0.89 M sucrose solution during water diuresis or

Fitzpatrick There is just a possibility that this discrepancy may be even larger in that your observations do not take into account the possible antagonism between vasopressin and oxytocin on myometrial response I have observed that a preliminary dose of vasopressin inhibits the response of the uterus to a subsequent dose of oxytocin in the bovine and in man Other authors have reported similar findings (Weinstein & Friedman, *Amer J Obstet Gynec* **29**, 93 1935)

Harris G W There are some observations which show that vasopressin inhibits the response of the rabbit s uterus to administration of oxytocin (Harris *J Physiol* **107**, 436, 1948 and *Neural Control of the Pituitary Gland* London, Edward Arnold) I believe the evidence indicates that this is an action on the blood vessels of the uterus rather than directly on the muscle cells

Dale There is a point of general physiological importance here How far are we to interpret the intra arterial injection of small doses of markedly hypertonic saline as giving a physiological stimulus? If they do we must conclude that the effect is not to produce as might teleologically be expected a preferential liberation of anti diuretic hormone but to produce, at the same time 20 or 30 times as much of the oxytocic hormone

Pickford I do not think one can call an intra arterial injection of sodium chloride physiological On the other hand this work raises the possibility that oxytocin itself has a physiological action on the kidney

Dale You cannot however be sure, I think that you are really reproducing the normal more gradual effect of a mild dehydration, such as we may get between meals or between drinks

Pickford If oxytocin does facilitate sodium excretion it could in mild dehydration tend to delay the onset of thirst

Heller In diabetes insipidus there are, so far as I know no very pronounced changes in the electrolyte metabolism In adrenal insufficiency on the other hand the disturbances are profound If the neurohypophysial hormones are of physiological importance one would expect some alterations in diabetes insipidus

Pickford One possibility is that oxytocin is concerned with rapid changes and not slow changes

Dale So far as evidence goes the antidiuretic effect that is to say the production of urine in the normal volume and concentration is the one function attributable to the neurohypophysis which is regularly in action and necessarily physiological Without it we become insipid diabetics If the effect of arterial hypertonic injections is really a proper representation of what is happening physiologically we have to accept I suppose, the fact that the neurohypophysis is putting out 20 to 30 times as much oxytocin as vasopressin And since by definition the balance in units between oxytocin and vasopressin in the gland remains something like 1 1 that must mean that the manufacture of oxytocin is going on 20 to 30 times as rapidly as that of vasopressin That would be an extraordinary and a profoundly important physiological conclusion

Pickford I think the results in the diabetic dog with a normal functioning kidney, showing that it did not respond to sucrose answered the last point of Dr Sawyer

Sawyer What was the urine flow rate?

Pickford Not higher than 1 5 ml /min I think it was about 1 0 ml per min The experiments of Keller *et al* are very interesting but they did use big doses and they did perform a terribly mutilating operation to produce diabetes and I don t see how they could avoid impairing anterior lobe function They admit that there were changes in the reproductive system and that the dogs were blinded Both these things suggest disturbance of anterior lobe function

de Wardener Is there an effect of oxytocin on cardiac output?

Pickford I know of no evidence of this I have seen no change in blood pressure after the intravenous injection of Pitocin in large doses into anaesthetized animals This, of course, gives no information on cardiac output

Chalmers I would like to report two observations on patients with diabetes insipidus in which we found that the resting clearances of inulin and p aminohippurate were not far below normal One patient a man aged 50, had an inulin clearance of 90 ml /min and a p aminohippurate clearance of 450 in the other a boy of 20, the clearances were 115 and 455 respectively We infused Pitocin at the rate of 1 mU per min for an hour—we perhaps should have used higher doses in view of Dr Pickford s observations—and in the case with the higher initial clearances there was no change whatever during or after the infusion In the other case during the infusion there was at first no change but later this patient showed a febrile reaction in which the PAH clearance rose from 450 to 1 300 without change in the inulin clearance

Croxatto We made observations on rats using a special preparation of Pitocin (prepared by Parke Davis and Co) which contained only a small amount of vasopressin (less than 3 per cent) In both normally hydrated rats and in hyper hydrated rats it elicited a diuretic response with a striking increase in sodium excretion In another series of experiments oxytocin was injected after having been exposed to the action of various proteolytic enzymes The diuretic and natriuretic effects, as well as the *uterotonic effect were destroyed by chymotrypsin but not by trypsin The doses of* oxytocin given were 25 mU and 50 mU per rat

Pickford I think it important to interpret changes in sodium excretion after oxytocin in terms of what we believe to be physiological doses

Garrod With regard to the striking difference in the amounts of vasopressin and oxytocin which Dr Pickford said were released I would ask her on what evidence this was based

Pickford That was based on the simultaneous observation of spontaneous uterine activity and urine flow during water diuresis in the same animal, after the intra carotid injection of 1 2 to 1 4 ml of hypertonic saline Matching the responses with oxytocin and vasopressin (Pitocin and Pitressin) required about 80 mU *Pitocin and* 3 mU Pitressin

The location of antidiuretic action in the mammalian kidney

by

H WIRZ

Physiologisches Institut der Universitat Basel

IT seems to be a well established fact that in the mammalian kidney the antidiuretic hormone acts chiefly by increasing the rate of water reabsorption so that the osmotic pressure of the urine is raised above that of the plasma With appropriate doses of the hormone full antidiuresis is obtained in most species without significantly altering filtration rate Some modifications of solute reabsorption may ensue but the increased urine concentration is due in the first place to the greatly diminished urine volume

Oddly enough in the absence of antidiuretic hormone the kidney is not producing what would seem to be the easiest thing to produce An isotonic urine in a normal kidney is the result of a moderate antidiuretic action With no antidiuretic hormone circulating in the body as in water diuresis urinary osmotic pressure is reduced well below isotonicity If therefore we want to approach the antidiuretic mechanism for instance by micropuncture studies we ought to start at the bottom of the problem investigating the conditions of water diuresis first

Unfortunately it is rather difficult to produce a good water diuresis in an anaesthetized and laparatomized animal The choice of the anaesthetic is one of the important factors Alcohol is one very good anaesthetic which in the rat does not interfere with water diuresis However it is not suited for the purpose for another reason it very appreciably depresses the freezing point yet on biological membranes it does not seem to exert an osmotic activity So any fluid containing alcohol may be biologically hypotonic whereas the physical determination seems to reveal hypertonicity Finally I got acceptable results with intravenous injections of Inactin which is a thiobarbiturate manufactured by Promonta (Hamburg)

Even with a perfect anaesthetic the laparatomy and the preparation of the kidney for micropuncture are very likely to interfere with water diuresis This difficulty has finally been overcome by applying the following technique (Wirz 1955 1956) The kidney of male rats is approached by a lumbar incision The adipose tissue is carefully removed from its dorsal face For the micropuncture the organ is supported by a little cup which by means of a stalk is firmly attached to the heated animal board The advantage of this method is that it avoids any handling of abdominal organs and that it affords an efficient immobilization of the kidney by simple means With this method osmotic *U/P* ratios of as little as 0 2 may be obtained

The procedure otherwise is very similar to that described by Walker & Oliver (1941) except that in the present investigation the Leitz Ultropak microscope was used instead of an ordinary binocular instrument Osmotic pressures of tubular

Pickford That is so, but as yet we do not know whether these effects are physiological or merely pharmacological

de Wardener When you say 20 to 30 times as much you refer to activity but is that any indication of the amount in milligrammes?

Dale That must mean almost the same thing, because, according to Professor van Dyke, pure oxytocin contains about 500 mU /mg and pure vasopressin about 600 mU /mg

de Wardener There is a further point The concentration of the urine is greater after dehydration than after the administration of vasopressin I wonder if this difference is due to oxytocin

Pickford One of the things we intend to do is to dehydrate ourselves and then try the effect of vasopressin, and then of vasopressin plus oxytocin, and see whether we can increase our urine concentrations up to the levels we get in dehydration Also Vogt (*Brit J Pharmacol* **8**, 193, 1953) has shown there is very little oxytocin in the hypothalamus of the dog and it all appears on the way down to the posterior lobe We thought of removing the posterior lobe and trying to rid the dog of all oxytocin and still have some vasopressin about

Cole I wonder if anyone has a measure of the rate at which administered oxytocin disappears from the circulation Heller and his co workers (Heller & Urban, *J Physiol* **85**, 502 1935 Ginsburg & Heller *J Endocr* **9**, 283, 1953) have shown that injected vasopressin is removed very rapidly and if the rate of removal of oxytocin is considerably slower it might help to account for the apparent high ratio of oxytocin/vasopressin in cases where the liberation of these hormones from the pituitary is inhibited

Heller That is an aspect in which we are interested but which we have not yet been able to investigate since the methods of estimating oxytocin in blood have not been adequate Bisset and Walker have recently improved the situation in this respect and they can perhaps help us

Walker By our method (*J Physiol* **126**, 588 1954) it is possible to measure oxytocin when added to blood and also we believe the level of endogenous oxytocin, but we have not yet tried estimating oxytocin in blood after administration of the commercial extract to the intact animal

Bernstein Has Dr Pickford considered whether any of her results could be explained as diurnal? Further has Dr Pickford any observations concerning the effect of variations in sodium intake?

Pickford Diurnal variation is eliminated since all procedures were done at the same time of day on each occasion We are currently investigating the variation of sodium intake in this respect but we have too few observations as yet to draw conclusions

van Dyke Was there any difference between the excretion of sodium and of chloride?

Pickford The chloride excretion was always qualitatively similar to that of sodium although perhaps not always of the same magnitude

different parts of the nephron is still somewhat arbitrary For convenience I under stand by distal convoluted tubule the segment which begins at the point where the ascending limb of the loop of Henle touches the glomerulus of the same nephron and which ends at the nearest bifurcation of the collecting duct This segment is probably not identical with the part of the nephron referred to as distal system by most physiologists

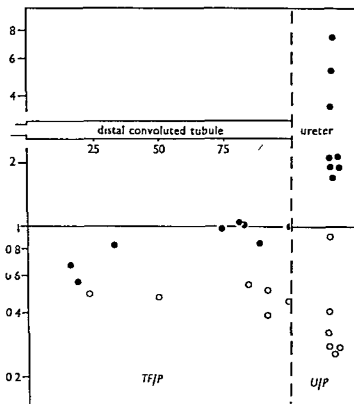


Figure 1

Distal tubular fluid/plasma ratios (*TF/P*) and ureteral urine/plasma ratios (*U/P*) of total molecular concentration in the concentrating (●) and the diluting (O) rat kidney The site of micropuncture is given as per cent of the length of the distal convoluted tubule (From Wirz 1956)

The results are shown in Fig 1 The open circles relate to experiments with animals in water diuresis and hypotonic urines the black dots to antidiuretic experiments with osmotic *U/P* ratios well above 1 0

The most striking fact is that in the concentrating kidney distal convoluted tubule fluid not only is not hypertonic but even very definitely hypotonic particularly in the beginning of this segment The results of Walker *et al* (1941) are thus fully con firmed Owing to the small number of successful distal punctures these authors were rather cautious as to the significance of their results In the light of the new facts those early findings are highly significant and so is the statement that it would appear that

fluid, ureteral urine and systemic plasma were determined by our microcryoscopic method (Hargitay Kuhn & Wirz 1951), which is similar to that described by Ramsay (1949) This method has a number of advantages over that of Barger (1904) hitherto used by micropuncture specialists Its accuracy is nearly as good as that of the Beckmann method on a macro scale The cryoscopy is performed in the same quartz capillary tube which previously had served for the micropuncture Therefore any evaporation or condensation which might occur during a transfer from one tube to another is *a priori* excluded Finally the amount of fluid required for cryoscopy is so minute that it can be drawn from any tubule at once This avoids prolonged collecting periods the blocking of the tubule by oil and the hazard of contaminating the sample with fluid from higher up or lower down the nephron

RESULTS

Proximal convoluted tubules

As to the location of the antidiuretic activity it has been presumed by most investigators of recent times that the proximal convoluted tubules have nothing to do with it Water reabsorption in this part of the nephron is regarded as a passive process secondary to an active reabsorption of solutes Water seems to follow *pari passu* the actively reabsorbed glucose sodium chloride, &c leaving behind an essentially isotonic tubular fluid

Experimental evidence so far has only been provided for the concentrating kidney by Walker Bott Oliver & McDowell (1941) but the same is also true for the diluting organ during water diuresis (Table I) The number of experiments is not very large

TABLE I

Proximal tubular fluid/plasma ratios (TF/P) and ureteral urine/plasma ratios (U/P) of total molecular concentration The site of puncture is given as per cent of the length of the proximal convoluted tubule

TF/P	*U/P*	Site (per cent)
1 01	0 41	20
1 00	0 25	28
0 99	0 20	30
0 97	0 20	37
0 99	0 26	42

but the results are so clear cut—the osmotic tubular fluid/plasma ratio was close to unity in every single experiment even at very low (ureteral) *U/P* ratios—that there is no need for extending these experiments further

Distal convoluted tubules

By the use of the Ultropak system it has become possible to distinguish quite definitely distal from proximal convoluted tubules *in vivo* The denomination of the

Those who are familiar with the matter will have noticed that the explanation I have given to the new findings is almost identical with that of H W Smith (1951) and of Wesson & Anslow (1952) From their clearance studies these authors have concluded that there must exist in the mammalian kidney three separate sites and three different mechanisms of water reabsorption This hypothesis has now been corroborated in its essentials, a few modifications and specifications may be added *on the strength of the new experimental facts The first process of water reabsorption* ($T^{p}_{H_2O}$ of these authors), amounting to approximately seven eighths of the filtered water, occurs in the proximal convoluted tubules It is visualized as an obligatory but passive process consequent to the proximal reabsorption of about seven eighths of the filtered solutes, leaving the tubular content essentially iso osmotic throughout the length of the proximal convolution This has now been demonstrated to be true not only for the concentrating but also for the diluting kidney—at least as far as the proximal convolution is accessible from the renal surface No sign of even slight hypo tonicity has been encountered in the present investigation It seems that osmotic *equilibrium is maintained all along the proximal convoluted tubules*

Secondly we have to assume a facultative water reabsorption ($T^{d}_{H_2O}$) in what Smith calls the 'distal system This part of the reabsorptive process is facilitated by the antidiuretic hormone It comprises the water osmotically liberated by distal sodium reabsorption (T^{d}_{N}) and if present, leads to an isotonic distal tubule fluid Hence it is probably a passive process too The present experiments demonstrate that $T^{d}_{H_2O}$ must be located in the convoluted part of the distal tubules This site is not quite identical with that of distal sodium reabsorption T^{d}_{N} apparently takes place already in the loop of Henle

Finally *there must exist a site of hyperosmotic water reabsorption* (T_{H_2O}) necessarily lower down in the nephron which abstracts water to concentrate the isotonic tubule fluid above the osmotic pressure of the plasma This hyperosmotic reabsorption must take place even beyond the nephron in the collecting ducts as no hypertonicity has ever been demonstrated to occur before the cortical branchings of the collecting ducts

There is however still at least one important point of controversy between New York and Basel left as a basis for discussion The ultimate process promoting T_{H_2O} is considered by H W Smith and his group to be located in the epithelium of the collecting ducts whereas we think that the collecting ducts play but a passive part and that the leading system is the hair pin counter current system provided by the loops of Henle

If water was removed from the collecting ducts by an active process located in the collecting duct epithelia, the interstitial fluid and the blood in the vasa recta of the papilla would have *to become hypotonic with respect to systemic blood This* hypotonicity might admittedly be very slight if the blood supply to the papilla was large as compared to urine flow But such a process could never induce the papillary blood to become hypertonic which according to micropuncture studies on the papilla of the golden hamster it is (Wirz 1953) Since these experiments are well known to some of you I can report them briefly The golden hamster has a long papilla pro truding into the ureter where it can be exposed *in vivo* It has been demonstrated that blood drawn by micropuncture from papillary blood vessels has practically the

the site of chloride reabsorption must be proximal to that of water. Water reabsorption however seems to catch up with the reabsorption of solutes at the end of the distal convolution. In most cases the tubular fluid is strictly isotonic throughout the last third of this segment. In one case isotonicity was not quite reached, but the tubular fluid/plasma ratio was never greater than 1.02. Thus there is no sign of an active process of water reabsorption up to the beginning of the collecting ducts.

In the diluting kidney on the other hand the hypotonicity of tubular fluid is maintained (perhaps even enhanced) throughout the length of the distal convoluted tubule. At the end of the segment the hypotonicity in most cases is not quite as pronounced as in ureteral urine. It is questionable however whether this points to a further solute reabsorption in the beginning of the collecting ducts. Alternatively, since ureteral urine is the composite product of some 30 000 nephrons, this finding may be due to a somewhat different diluting capacity of different renal units.

In short it may be stated that the diluting process occurs before and possibly along the distal convolution. It is completed, or nearly so, at the beginning of the collecting ducts. The concentrating process on the other hand must in some way include the collecting duct system.

DISCUSSION

Apart from the possibility of water secretion in the distal segment, which would have to occur even in antidiuresis and for which I do not see any reason, the hypotonicity of the tubular fluid at the beginning of the distal convolution shows that there must *take place between the end of the proximal and the beginning of the distal convolution* a copious active reabsorption of one or more solutes without or with less than an equivalent amount of water. For lack of other evidence we may tentatively assume that this isolated solute reabsorption is located in the ascending limb of the loop of Henle.

As to the substance or substances whose reabsorption causes the hypotonicity of distal tubule fluid I agree with H. W. Smith (1951) and with Wesson & Anslow (1952) that the only solutes available for reabsorption in sufficient amount are sodium, conceivably the active part, and chloride as a concomitant anion. These experiments may be taken as a factual demonstration—and to some extent location—of what these authors call T^d_{Na}. Along the segment concerned with distal sodium reabsorption poor water permeability must exist in both the diuretic and the antidiuretic state. But only in water diuresis does this impermeability obtain throughout the entire distal convolution. By the action of the antidiuretic hormone the distal convoluted tubules apparently become permeable to water, allowing the tubular content to become isotonic with the surrounding interstitial fluid. There is no sign however of an *active* water reabsorption in the distal convoluted tubules. Even in severe antidiuresis at (ureteral) U/P ratios as high as 7 the cortical tubules show an isotonic content at best.

In the absence of antidiuretic hormone on the other hand, the epithelial walls must be virtually impermeable to water not only in that part of the loop and the distal convolution where hypotonicity is produced but also in the collecting ducts, where the hypotonicity is maintained. Thus only is it explainable that the urine leaves the kidney in the same hypotonic state which it already attained in the distal convolution.

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same osmotic pressure as the urine which is formed simultaneously The experiments include osmotic U/P ratios between 1 0 and 2 It has been concluded from these experiments that in the concentrating kidney the whole of the papilla becomes hypertonic including the contents of the collecting ducts and the extracellular fluid

Earlier experiments (Wirz, Hargitay & Kuhn 1951), using the method of direct cryoscopy of kidney slices had led to the conclusion that the contents of both limbs of the loops of Henle also were hypertonic in the papilla of concentrating kidneys From these results the course of osmotic pressure along the nephron and collecting duct was outlined as in Fig 2 The objection has been made that the results of direct

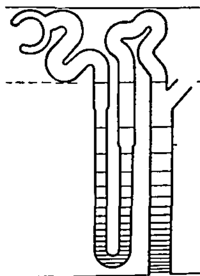


Figure 2

Variation of osmotic pressure in a single nephron and collecting duct as computed from direct cryoscopy of the rat kidney (From Wirz *et al* 1951)

cryoscopy might be partly influenced by diffusion *post mortem* Apparently these experiments failed to disclose the slight hypotonicity at the beginning of the distal convoluted tubules of concentrating kidneys which was demonstrated by micro puncture studies But even if the cryoscopic readings showed nothing but an average freezing point depression in any single slice, they would be conclusive at least in one respect Freezing points as low as $-4\ 3°$ C have been recorded in the papillae of antidiuretic rats It is very improbable that so high an average osmotic pressure could be the result of a mixture of the hypertonic content of the collecting ducts and of the isotonic contents of loops blood vessels and interstices

A different line of approach was followed by Ullrich Drenckhahn & Jarausch (1955), who investigated the osmotic behaviour of slices taken from various depths of fresh dog kidneys By extrapolation they computed the osmotic pressure of a saline solution with which the slices would be in osmotic equilibrium In concentrating kidneys they found a steady increase of osmotic pressure from the cortico medullary

boundary towards the tip of the papilla These results are significant in two respects Firstly as it is the cells that will shrink or swell they demonstrate that the intra cellular compartment too is involved in the osmotic stratification of the renal medulla Secondly the last slices in all experiments on concentrating kidneys show within the limit of methodical errors, the same osmotic pressure as the urine which was formed immediately before the animal was killed Thus the epithelial cells of the collecting ducts seem to be in perfect osmotic equilibrium with the urine within

The same authors found the sodium concentration of such slices too to increase with the depth of the renal medulla Final concentrations of up to 425 mEq sodium per litre of tissue water were found, about four times the sodium concentration of the urine This suggests the existence of high ionic concentration gradients between the different compartments combined with an absence of high total molecular con centration gradients

The results of these workers recall the earlier experiments of Ljungberg (1947) who demonstrated an increase of chloride concentration in the medulla of rabbit kidney Here again the average chloride concentration of slices from the deeper parts of the medulla was well above that of both the urine and systemic plasma or whole blood According to Ljungberg s interpretation these findings indicated an active chloride reabsorption from the collecting ducts He assumed that the chloride con centration of the extracellular fluid was the same in the renal medulla as in other parts of the body An alternative view was offered by H W Smith (1951) namely that a high intracellular concentration of chloride might be involved in the re absorption of water to form a hypertonic urine He suggested that water might diffuse into such an intracellular system by osmotic pressure Assuming an interstitial fluid of normal osmotic composition this interpretation would leave the question open how the water, once in the epithelial cells would be disposed of

THE HAIRPIN COUNTER CURRENT SYSTEM

There is only one explanation which covers all the known facts and moreover gives a satisfactory reason for the existence of the loops of Henle in the mammalian kidney This theory is based on the combination of a flow along a tube that doubles back upon itself (hairpin counter current) and an active process setting up a (small) osmotic pressure difference between the two limbs of this tube the content of the descending limb being (slightly) hypertonic The concentrating effect of this system starts being multiplied as soon as the (slightly) hypertonic content of the descending limb is carried by bulk flow around the bend into the ascending limb The lower ends of both limbs now being equally hypertonic the osmotic pressure difference between the two limbs is momentarily abolished If the active osmotic process continues a new osmotic pressure difference is set up the content of the lower part of the descending limb now being twice as hypertonic as in the first step This fluid is again transported into the ascending limb again abolishing the osmotic pressure differences between the two limbs and so on Finally a steady state arises the fluid entering the system at the upper end of the descending limb being more and more concentrated towards the hairpin bend and rediluted on its way back up the ascending limb Osmotic pressures many times higher than the initiating single effect may be produced

The theory was developed both mathematically and experimentally with the help of a working model by Hargitay & Kuhn (1951) The primary difference in osmotic pressure in the model was brought about by a head of hydrostatic pressure applied to the descending limb both limbs sharing a common membrane of semipermeable material The significance of this model has often been misjudged It was not meant to demonstrate by it that the renal concentrating mechanism might be operating by means of physical forces alone The hydrostatic pressure was chosen by Hargitay & Kuhn as a simple and well defined driving force to produce a small osmotic pressure difference across a semipermeable membrane The model was to show that this small osmotic single effect may be multiplied if applied to a counter current system, resulting in final osmotic pressures several times higher than the single effect alone

It is not likely that in the kidney the driving force is a hydrostatic pressure difference as in the model The osmotic pressure difference between the descending and the ascending limb of the loop of Henle is probably brought about by an active cellular transport mechanism, either driving water from the descending limb across the interstices into the ascending limb or by a process moving one or more solutes in the opposite direction—or a combination of both

In any case the result of the counter current mechanism would be a milieu of increasing hypertonicity towards the tip of the renal papilla Besides the loops of Henle two other systems are passing through the same milieu The vascular system is anatomically arranged as a number of hair pin tubes extending more or less deeply into the medulla All the blood irrigating the medulla enters and leaves the medulla at the cortico medullary boundary i e in an essentially isotonic region In between it may adapt itself to the osmotic pressure of the surroundings by a passive uptake (on its way down) and release (on its way up) of osmotically active solutes Hence the finding of hypertonic blood by micropuncture of the papilla of the golden hamster

The second system passing through the medulla is that of the collecting ducts Here too a passive adaptation to the hypertonic milieu is assumed, not by an uptake of solutes but by a passive transfer of water according to the osmotic pressures Unlike the vasa recta the collecting ducts do not turn back to the cortex, and this urine concentration is final

The precise nature of the process initiating the counter current concentrating mechanism must be left open to further investigation The present results however may help by giving a *tentative* description of the antidiuretic mechanism in the mammalian kidney

The distal sodium reabsorption (T^{d}_{N}) might serve a double purpose Firstly the removal of a large part of what sodium is left over by the proximal system, and secondly the facultative initiation of the counter current concentrating mechanism If an active sodium transport was at work all along the ascending limb of the loop—and the present results seem to point in this direction—the extracellular fluid of the medulla would as a first step, become slightly hypertonic This alone would not suffice to get the concentrating mechanism going For this the crucial point is the entering at the hairpin bend of a (slightly) hypertonic solution into the ascending limb In other words, the contents of the descending limb would have to be granted osmotic equilibration with their (slightly) hypertonic surroundings

One might assume that in the absence of antidiuretic hormone the descending limb is impermeable to both water and solutes Its content would then stay isotonic as it was at the end of the proximal convolution In this case renal function would proceed as if there was no counter current system, distal sodium reabsorption combined with a watertight distal convolution and collecting duct, leading to large volumes of hypotonic urine

The osmotic equilibration of the descending limbs with their surroundings might be facilitated by the antidiuretic hormone by opening pores to allow a passive transfer

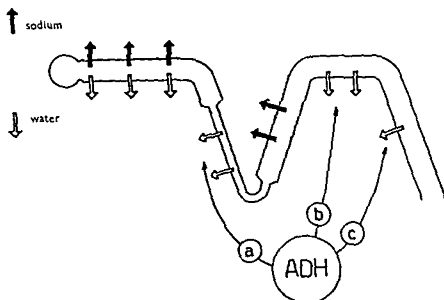


Figure 3

The action of the antidiuretic hormone is suggested as facilitating a passive transfer of water at the different sites in the nephron and collecting duct (for details see text)

of water (Fig 3 *a*) or sodium (chloride) or both If the pores were opened for water we would have to assume yet another site of facultative water reabsorption in the descending limb of the loop This water would combine with the sodium (chloride) turned out by the ascending limb and be carried away by the medullary blood If the pores were opened wide enough to allow an inward movement of sodium (chloride) we would have to assume a recirculation of part of this salt through the loop

This hypothesis has the advantage that no other function would have to be attributed to the antidiuretic hormone than that of opening the pores to allow a passive transfer of water (and possibly some solutes) It was first shown by Sawyer (1951) that in the frog skin the action of postpituitary extracts consists in lowering the barrier to osmotic entrance of water rather than in promoting any transport activity Also Koefoed Johnsen & Ussing (1952) concluded from tracer experiments on the

isolated toad skin that the action of neurohypophysial hormones could be explained entirely by an increase in pore size in some layer of the skin Although the active principle influencing the water uptake by the anuran skin is possibly not identical with the (mammalian) antidiuretic hormone, it seems to be an attractive hypothesis that the same purpose—the conservation of body water—is achieved by the neuro hypophysis in mammals and amphibians by influencing very different tissues but in essentially the same manner

In the mammalian kidney the sites of such action would be *a*, (see Fig 3) the descending limb of the loop allowing the tubular fluid to reach equilibrium with the hypertonic extracellular fluid and thus inducing the counter current concentrating mechanism *b*, the distal convoluted tubules, allowing water reabsorption to catch up with distal sodium reabsorption and finally *c*, the collecting ducts, where a passive transfer of water to the hypertonic surroundings would concentrate the urine above isotonicity

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Discussion

Chairman H Heller

Heller I should like to congratulate Dr Wirz on his elegant work I think I am right in saying that the only other workers who have succeeded in doing micropunctures in the mammalian kidney were Walker and his group and you of course have been able to get a great deal more information about the distal tubule

de Wardener I want first to add my own congratulations to Dr Wirz The observation that has delighted me most is your finding that when the urine in the ureter is hyper tonic that in the distal tubule is hypotonic As you said Walker and his co workers also obtained similar results on three occasions but I think I am right in saying that in these experiments the fluid in the distal tubule was not as hypotonic as in your own My pleasure in this finding is that it fits so admirably with the results of some of our own experiments

McCance (*J Physiol* (*Lond*) **104,** 196 1945) first showed and this has been con firmed on many occasions that in a dehydrated man or animal the administration of a substance which has then to be excreted in the urine causes not only a rise in solute output but a simultaneous increase in urine flow and a fall in urine concentration towards that of plasma It has been suggested that these changes are due to an in creased flow of iso osmotic tubular fluid into that site in the tubule where concentration takes place so that the concentration of the urine is now approaching that of plasma There is the assumption in this explanation that such an osmotic diuresis initiated at any level of hypertonicity however minor should be accompanied by a fall in urine osmolarity towards plasma

Dr del Greco and I however have recently demonstrated that this assumption is not warranted and that in fact if an osmotic diuresis is initiated in man when the urine osmolarity is only slightly above that of plasma there is a fall in urine osmolar ity below that of plasma the urine becoming hypotonic (del Greco & de Wardener *Clin Sci* **14,** 715 1955) To explain this phenomenon it seemed reasonable to suggest that when the urine is hypertonic there is somewhere in the tubule a site where the tubule fluid is hypotonic—a hypothesis first put forward by Shannon (*J exp med* **76,** 371 1942) It appeared therefore that the concentration of the urine not only depends on the level of circulating antidiuretic hormone and its action on the collect ing tubules but also on the rate at which hypotonic fluid is being delivered to this site

If this was correct it followed that it should be possible to make a hypotonic urine hypertonic by reducing the solute output This we have found is a correct deduction (del Greco & de Wardener *J Physiol* (*Lond*) **131,** 307 1956) We performed the experiment in animals made resistant to the effect of Pitressin by large and rapid ex pansions of the extracellular volume (Wesson Anslow Raisz Bolomey & Ladd *Amer J Physiol* **162,**677, 1950) and which were therefore passing hypotonic urine Changes in solute output were produced by aortic occlusion by means of an indwelling aortic inflatable rubber balloon Upon inflating the balloon the solute output fell and the urine became hypertonic upon deflating the balloon the urine again became

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of about 50 grammes and golden hamsters grow so quickly I have obtained a species of desert rat *Meriones shawi* which has a papilla similar to that of the golden hamster But what I have been doing up to now is breeding them

Sawyer We have another species of desert rat the Mongolian gerbil *Meriones unguiculatus* which also has a long thin papilla and presumably long collecting ducts This species is capable of living without weight loss on a diet of dry oatmeal without water The closely related African gerbil *Gerbillus gerbillus* has recently been reported by Burns (*Endocrinology* 58, 243 1956) to be capable of surviving on dry grain as can the kangaroo rat (*Dipodomys*) and the jerboa A number of desert rodents such as *Dipodomys*, *Meriones Gerbillus Jerboa* and *Perognathus* also have long renal papillae as Sperber (*Zool Bidr Uppsala* 22, 1944) has pointed out The gerbil breeds very easily However, the man who is breeding them commercially in the U S will only let us have males

hypotonic within 1–3 min Dr Sawyer tells me that Dr Berliner has recently completed almost identical experiments and has obtained very similar results

I think these observations fit very nicely with the findings of Dr Wirz that, somewhere in the tubule, the urine is at all times hypotonic

Heller There is a question which I might ask Dr Wirz and Dr Sawyer at the same time what in your present schemes is the significance of the thin loop of Henle? Homer Smith has suggested that it might be an equilibrating region—where iso osmoticity is obtained Do you agree with this concept?

Wirz Homer Smith suggested that proximal tubule fluid might, at times at least, be hypotonic This does not seem to follow from the experiments of Walker *et al* They however have worked exclusively on concentrating kidneys and many of their experiments were done during osmotic diuresis In my series I have never seen a hypotonic proximal tubule fluid even at low ureteral urine osmotic pressures, and without the use of osmotic diuretics I therefore do not see any need for further equilibration in the thin limb

Sawyer There are two data I want to get clear about in connexion with several criticisms which have been levelled against your work You do not think that in your sections there could be post mortem diffusion of water because your freezing point depressions were so high Is that right?

Wirz I don t see how a freezing point depression of more than 4 C could be attributed to a mixture of an isotonic fluid and one which in this case would have to be much more hypertonic A freezing point depression of 4 C nearly corresponds to the osmotic ceiling of the rat

Sawyer Would you also consider the possibility that the high osmotic pressure could be due to the hydrolysis of intracellular substances such as ATP which are described by Conway (Conway, Geoghegan & McCormack, *J Physiol (Lond)* **130**, 427 1955) as occurring in renal tissue even at 0° C ?

Wirz In cortical tissue slices we always found freezing point depressions close to 0 56° C which you would expect from isotonic fluids Furthermore this objection would certainly not apply to the findings—by other workers—of high sodium and chloride levels

Sawyer You have determined the osmotic pressure of blood taken from renal vessels in the living animal Is the blood flowing when you draw these samples?

Wirz Yes

Pickford There is one question I would like to ask about the blood vessels Do you ever see any change in the size of the blood vessels during the effect of antidiuretic hormone? Can you judge the size of the blood vessels which run parallel to Henle s loop? I think it has been suggested that constriction or dilation of these vessels is one of the factors involved

Wirz This is one thing I have not managed to do but which ought to be investigated Golden hamsters are very awkward You can only do these experiments with animals

The antidiuretic action of neurohypophysial hormones in Amphibia

by

WILBUR H SAWYER

Department of Physiology New York University College of Medicine and the Mount Desert Island Biological Laboratory

THE importance of the neurohypophysis in the regulation of the water metabolism of mammals is well known However an increasing body of evidence reveals that the neurohypophysial hormones are also concerned with water balance in the Amphibia These hormones act not only on the skin but also on the kidney and urinary bladder I intend to speak principally about neurohypophysial action on the kidney and will argue that the antidiuretic response in this organ resembles that in amphibian skin with respect to the fundamental mechanism of action Because the skin is readily accessible to direct study the action of neurohypophysial hormones on this organ has been the subject of numerous studies which have afforded us considerable insight into the mechanisms involved

Neurohypophysial hormones markedly increase the rate of water uptake through the skin of the frog and toad Here water uptake appears to be a passive process activated by the osmotic gradient between the body fluids and the external medium (Sawyer 1951*a*) The skin however does not behave like a simple homogeneous semipermeable membrane The net influx of water is several times that predicted from the measurement of total flux if it is assumed that the entire area of the skin is available to diffusion and that influx and outflux are directly proportional to water concentration on the respective sides of the skin (Hevesy Hofer & Krogh 1935 Capraro & Bernini 1952) Koefoed Johnsen & Ussing (1953) point out that it is unnecessary to invoke active water transport to explain this discrepancy if instead the skin is conceived as a system in which osmotic flow occurs through minute pores Neurohypophysial hormones increase the net flux of water without proportionately increasing the total flux (Capraro & Bernini 1952) This is most readily explained by assuming that the hormones dilate the pores

Professor Ussing at the Colston Research Society Symposium two years ago adduced substantial evidence in support of his pore theory from an examination of the permeability of toad skin to isotopically labelled thiourea (Ussing 1954) The thiourea molecule is considerably larger than water and normally penetrates toad skin at a slow rate Neurohypophysial hormone increases the rate of flux in both directions the increase in flux of thiourea being much greater than the increase in the flux of water This is best explained by assuming that the neurohypophysial hormone dilates pores that are normally barely large enough to admit water but too small to admit thiourea The influx of thiourea is more rapid than the outflux even when the

concentration is the same on both sides, but this phenomenon, in Ussing's view merely reflects the fact that thiourea is swept through the pores in the stream of the osmotic inflow of water

The pore theory has been supported by Garby & Linderholm (1954) on the basis of the effects of aminophylline on the relative rates of flux of urea and electrolytes through frog skin

The pore theory suggests a possible interpretation of how neurohypophysial principles may act on other semipermeable membranes No data previously available indicate whether it is applicable to the kidney, but the evidence presented here points in that direction

The older view in mammalian physiology held that the neurohypophysial anti diuretic hormone acts on the mechanism responsible for the elaboration of hypertonic urine On this basis we once believed that the change in the permeability of frog skin could not share a common mechanism with the response of the mammalian nephron (Sawyer 1951*a*) But we now have reason to revise this opinion radically On recent evidence Professor Homer Smith (1956) has argued that the antidiuretic hormone may not be necessary for the activation of the concentrating mechanism, an interpretation which agrees with the observation that hypertonic urine can be produced in the apparent absence of this hormone (Shannon, 1942, Levkoff Demunbrun & Keller, 1954, Berliner & Davidson, 1956)

Turning to the amphibian kidney where the urine is never hypertonic to the blood neurohypophysial extracts are known to increase the tubular reabsorption of water (Pasqualini 1938 Sawyer 1955 Jancsó, 1955*a*, 1955*b* Sawyer, 1956) Here we have an opportunity to examine neurohypophysial action on the nephron in the absence of a mechanism for elaborating a hypertonic urine

Two large amphibians, the North American bullfrog *Rana catesbiana* and the South American toad, *Bufo marinus* have provided material for these studies The basic technique consisted of cannulating both ureters through a ventral abdominal incision with fine polyethylene tubing The tubing was brought out through the cloaca, the abdomen closed and the animal allowed to recover from anaesthesia The next day ureteral urine was collected continuously from the unanaesthetized animal Ureteral cannulation has the advantages of eliminating most of the dead space and any errors due to reabsorption through the bladder

This preparation allows one to make multiple consecutive determinations of exogenous creatinine or inulin clearances These clearances when determined simultaneously in our preparations are essentially identical and are assumed to be valid measurements of filtration rate (Forster 1938 Sawyer & Sawyer 1952 Sawyer, unpublished) The osmotic concentrations of plasma and urine were deter mined by freezing point (Wesson 1952) or vapour pressure (Sawyer 1954) depres sion These data enable us to calculate the osmotic and free water clearances respectively (Smith 1956) The osmotic clearance ($C_{osm} = U_{osm}V/P_{osm}$) is defined as that portion of the total urine flow required to contain the urinary solutes in an *isosmotic solution* The free water clearance ($C_{H_2O} = V - C_{osm}$) is the water excreted in excess of that which is obligated osmotically This is the water that dilutes urine below the isosmotic state

Neurohypophysial extract administered in large doses to the bullfrog causes a

marked decrease in free water clearance, even when this is factored by the filtration rate to eliminate variable changes in this function (Fig 1) This reduction in free water clearance leads to antidiuresis and to an increase in the osmotic concentration of the urine this concentration approaching that of the plasma Tubular antidiuresis is irregularly accompanied by a decrease in filtration rate (Fig 2) which we have previously described as the glomerular antidiuretic response (Sawyer 1951*b* Sawyer & Sawyer 1952) We believe that the glomerular action is attributable to specific constriction of the afferent glomerular arterioles by the neurohypophysial hormones

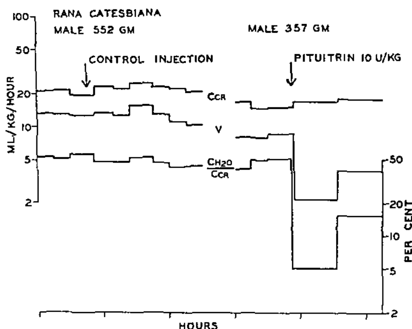


Figure 1

Antidiuret c respon e of the bullf og to mammalian neu ohypophys al extract The control injection was dilute acet c acid of the same volume and pH as the P tuitrin solution In this example the extract did not depress the filtration ate (Ccr) The reduction in urine flow (V) was due to the d pression of the elative f e wate lea ance (C_{H_2O}/C_{cr})

The responses of the toad to neurohypophysial extract are similar to those of the bullfrog The change in filtration rate is variable as in the frog and the antidiuresis is attributable primarily to reduction in free water clearance (Sawyer 1956)

Similar changes in urinary concentration and filtration rate have been described in the bullfrog (Schmidt Nielsen & Forster 1954) and toad (Sawyer 1956) in response to dehydration but the data are insufficient to implicate the neurohypophysis We have however demonstrated depletion of hormone in the frog neurohypophysis during dehydration (Levinsky & Sawyer 1953), an observation which has been recently confirmed by Jancso (1955*a* 1955*b*) and we infer that dehydration evokes release of neurohypophysial hormone in the frog

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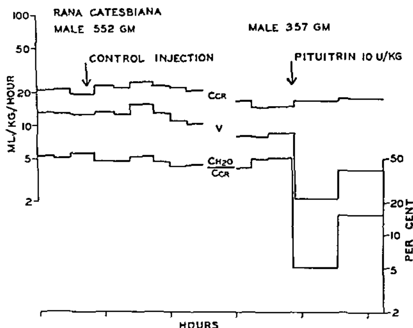


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Moreover, there is clearly enough hormone present in the amphibian neurohypophysis to produce antidiuresis An injection of bullfrog pituitary extract corresponding to 1/100 of a gland produces marked antidiuresis in this animal (Fig 3) This

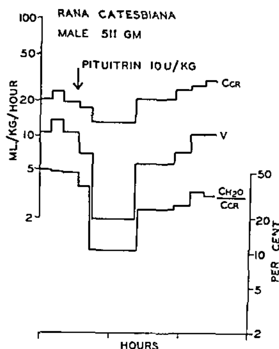


Figure 2

Antidiuretic response of the bullfrog to mammalian neurohypophysial extract In this instance the extract caused a decrease in filtration rate The reduction in free water clearance however accounts for the greater part of the antidiuresis

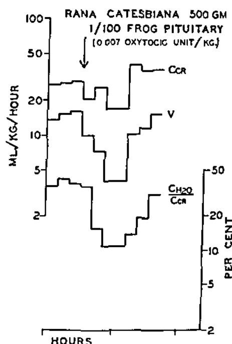


Figure 3

Antidiuretic response of the bullfrog to the injection of frog neurohypophysial extract equivalent to 1/100 of a gland This amount of extract corresponded to a dose of 0 007 oxytocic u /kg

extract, when assayed on the rat uterus contained only 0 4 oxytocic units per gland The effective antidiuretic dose in the frog was, therefore, only 0 007 rat oxytocic units/kg It takes five to ten oxytocic units/kg of mammalian extract to produce a comparable antidiuretic effect A similar difference in the effectiveness of amphibian and mammalian extracts with respect to the frog water balance response has been demonstrated by Heller (1941)

Similarly, a dose of toad pituitary extract corresponding to 1/100 of a gland produced as great an antidiuresis in the toad as one unit of mammalian extract (Sawyer 1956)

This discrepancy implies class specificity among the active neurohypophysial principles There is a difference with respect to one amino acid between vasopressin from the ox and hog (Popenoe Lawler & du Vigneaud, 1952), and as Heller (1955) has implied, a greater difference between mammalian and amphibian neurohypophysial principles would not be surprising The fact that oxytocin is more effective than is vasopressin in producing water balance (Heller, 1930) and antidiuretic

(Sawyer 1951*b* Sawyer & Sawyer 1952) responses in the frog suggests that oxytocin resembles the frog neurohypophysial hormone more closely than does vasopressin (Sawyer 1955)

That a physiologically active contaminant of the neurohypophysial extract does not account for the difference in antidiuretic activity of these extracts in the bullfrog seems to be excluded by the fact that synthetic oxytocin (supplied by Dr Vincent du Vigneaud) per oxytocic unit is just as active as whole mammalian extract in producing an antidiuresis (Fig 4)

Precarious as it may be to do so we may venture to diagram the possible action of neurohypophysial hormone on tubular water reabsorption in the Amphibia (Fig 5)

If we agree that urinary water is wholly derived from the glomerular filtrate * it follows that osmotically free water in the urine is formed by the reabsorption of solute (chiefly NaCl) from this filtrate leaving water behind for excretion in the urine This requires a tubular segment capable of active solute reabsorption but relatively impermeable to water In the Amphibia the renal tubule must be almost impermeable to water throughout its length during water diuresis since the excretion of osmotically free water may amount to 50 per cent of the filtered water

We need only suppose that neurohypophysial hormones increase the permeability of the tubule to water to explain the observed changes in free water clearance Jancso (1955*a*, 1955*b*) has confirmed the increase in total water reabsorption by the frog renal tubule under the influence of neurohypophysial hormones and derived evidence on the anatomical location of the reabsorptive process by observations on the excretion of trypan blue and non reabsorbable polyvinyl polymers During antidiuresis these substances are deposited as casts in channels occurring only in a specialized segment of the terminal distal tubule immediately before it joins the collecting duct and, to a lesser extent in the collecting ducts themselves

We have noted that the skin of the frog and toad shows variable permeability to water Neurohypophysial extracts and synthetic oxytocin (Sawyer unpublished) increase this permeability probably by increasing the size of pores (Ussing 1954) We have recently found that neurohypophysial extracts (Schisgall & Sawyer, 1956) and synthetic oxytocin (Sawyer unpublished) also increase water reabsorption from the frog urinary bladder an organ ordinarily not included in discussions of water balance There is evidence, however that water in the amphibian bladder can be reabsorbed (Steen 1929 Ewer 1952*a* Schisgall & Sawyer 1956)

The probability that the responses of the renal tubules skin and bladder involve a common mechanism is brought out by a comparison of the effects of neurohypophysial hormones on these organs in frogs and toads (Table 1) In all three instances permeability to water is increased by dehydration and amphibian extracts are more effective than mammalian extracts In the frog the response is greater with oxytocin while in the toad the response is greater with vasopressin All these phenomena may depend on the dilatation of pores though there is no evidence for the pore theory except in the case of the skin

* In view of the fact that the frog tubule can secrete urea and other solutes *we cannot deny that some water* may enter the urine accompanying such solutes (Schisgall Buxer & Sawyer 1956) It is also apparent that water flux will occur in both directions between tubular urine and the interstitial fluid if the tubule is at all permeable to water In this argument we are however concerned only with net flux

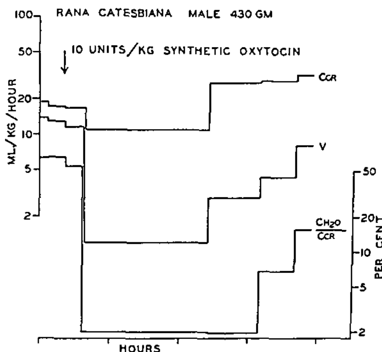


Figure 4
Antidiuretic response of the bullfrog to the injection of synthetic oxytocin

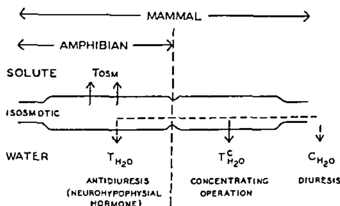


Figure 5
Schemat c representation of the relationship of solute reab orptı n ($T_{\mathrm{o\,m}}$) to the formation of free water ($C_{\mathrm{H\,O}}$) in mammal and am phibian in diuresis and antidiures s Neurohypophys al horm ne is indicated as acting to allow the pass ve real sorpt on ($T_{\mathrm{H\,O}}$) of water f eed o motically by solute r absorption The portion of the diagram labelled amph bian is suppo ed to include the entire nephron The portion labelled mammal is int nd d to represent only the nephron distal to the thin s gment

TABLE I

Properties of the change in water permeability of skin bladder and renal tubule of frogs and toads

	Animal	Increase in water permeability		
		Skin	Bladder	Renal tubule
Occurs in dehydration	Frog	+(1)*	+(2 3)	+(4)
	Toad	+(5)	+(6)	+(7)
Amphibian pituitary extract more active than mammalian	Frog	+(8 9 10)	+(9)	+(11 12)
	Toad	+(13)		+(7)
Oxytocin more active than vasopressin	Frog	+(10 14 15)	+(9)	+(10 16)†
Vasopressin more active than oxytocin	Toad	+(17 18 19)	+(5)	+(18 19 20)†
Synthetic oxytocin active	Frog	+(9)	+(9)	+(11)

Species and references as indicated by numbers in parentheses

(1) *Rana pipiens* Levinsky and Sawyer 1953
(2) Frog Probably *R pipiens* Steen 1929
(3) *R catesbiana* Schisgall & Sawyer 1956
(4) *R clamitans* Schmidt Nielsen & Forster 1954
(5) *Bufo regularis* Ewer 1952*b*
(6) *B regularis* Ewer 1952*a*
(7) *B marinus* Sawyer 1956
(8) *R temporaria* Heller 1941
(9) *R pipiens* Sawyer unpublished
(10) *R temporaria* Jørgensen 1950
(11) *R catesbiana* Sawyer this paper
(12) *R esculenta* Jancsó 1955*a* 1955*b*
(13) *B bufo* Jørgensen 1950
(14) *R esculenta* Heller 1930
(15) *R pipiens* Sawyer Travis & Levinsky 1950
(16) *R catesbiana* Sawyer & Sawyer 1952
(17) *B arenarum* Novelli 1932
(18) *B regularis* Ewer 1951
(19) *B carens* Ewer 1952*b*
(20) *B marinus* Sawyer & Sawyer 1952

† Total antidiuretic effect measured not tubular effect alone

The interpretation given here for the function of neurohypophysial hormones in the Amphibia brings this function into line with the present interpretation of the action of the antidiuretic hormone in the mammalian nephron (Fig 5) It is supposed by most investigators that in the mammal about 80 per cent of the filtered solute is reabsorbed by the proximal tubule the free water formed by proximal solute reabsorption diffusing back into the blood either here or through the thin segment (Smith 1951) so that the urine delivered to the distal segment‡ is again isotonic with the blood though reduced in volume by four fifths Free water becomes available for excretion in the distal tubule where most of the remaining sodium is actively reabsorbed (Wesson & Anslow 1952 Smith 1952) During water diuresis this free water diffuses so slowly through the relatively impermeable epithelium of the distal tubule that it escapes reabsorption Up to this point the mammalian distal tubule bears a close resemblance to the amphibian tubule

In the mammal however during antidiuresis the urine may be concentrated to an osmotic pressure above that of the plasma an operation which involves directly

‡ The terms distal segment as used here indicates a functional portion of the nephron between the thin segment and the concentrating mechanism as defined below but cannot be identified as yet with any specific anatomical portion of the distal tubule or connecting tubule in the Amphibia or mammals

or indirectly, the active reabsorption of water in contradistinction to the passive diffusion with which we have been concerned up to this point Reasons have been given for believing that this concentrating operation ($T^c_{H_2O}$) is physiologically and anatomically distinct from the mechanism of water diuresis (Wesson & Anslow, 1952, Smith, 1951, 1952, 1956) This concentrating operation may or may not be continuous, but it removes water from the urine subject to two limitations it is limited by a maximal rate (ml /min) when the volume of urine delivered to it is large, while at lower volumes it is limited to a maximal osmotic U/P ratio Berliner & Davidson (1956) have recently reported that during acute reduction of filtration rate in one kidney in hydrated dogs otherwise showing maximal water diuresis, the urine may be concentrated to an osmotic level above that of the plasma, implying that the concentrating operation proceeds independently of the presence of the antidiuretic hormone

In this view the degree of urinary concentration in the mammal is determined therefore largely by the volume of water delivered to this concentrating mechanism We do not know how this concentrating mechanism acts, but reasons have been given for believing that this concentrating mechanism is located in the collecting ducts (Smith, 1956) If it is supposed that the antidiuretic hormone increases the permeability of the distal segment to water then during antidiuresis the volume of water delivered to the concentrating mechanism is reduced and the urine is correspondingly more concentrated by the operation of the latter As matters stand, diuresis and antidiuresis can then be explained in the mammal simply in terms of an increase in permeability of the distal segment to water under the influence of the antidiuretic hormone This increase in permeability since it involves reabsorption of water only up to the isosmotic state, could be interpreted in terms of a change in pore size as the evidence indicates to be the case in the skin, and as we have inferred to be the case in the frog bladder and the amphibian nephron

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or indirectly, the active reabsorption of water in contradistinction to the passive diffusion with which we have been concerned up to this point Reasons have been given for believing that this concentrating operation ($T^c_{H_2O}$) is physiologically and anatomically distinct from the mechanism of water diuresis (Wesson & Anslow, 1952, Smith, 1951, 1952, 1956) This concentrating operation may or may not be continuous, but it removes water from the urine subject to two limitations it is limited by a maximal rate (ml /min) when the volume of urine delivered to it is large, while at lower volumes it is limited to a maximal osmotic U/P ratio Berliner & Davidson (1956) have recently reported that during acute reduction of filtration rate in one kidney in hydrated dogs otherwise showing maximal water diuresis, the urine may be concentrated to an osmotic level above that of the plasma, implying that the concentrating operation proceeds independently of the presence of the antidiuretic hormone

In this view the degree of urinary concentration in the mammal is determined therefore, largely by the volume of water delivered to this concentrating mechanism We do not know how this concentrating mechanism acts but reasons have been given for believing that this concentrating mechanism is located in the collecting ducts (Smith, 1956) If it is supposed that the antidiuretic hormone increases the permeability of the distal segment to water then during antidiuresis the volume of water delivered to the concentrating mechanism is reduced and the urine is correspondingly more concentrated by the operation of the latter As matters stand, diuresis and antidiuresis can then be explained in the mammal simply in terms of an increase in permeability of the distal segment to water under the influence of the antidiuretic hormone This increase in permeability since it involves reabsorption of water only up to the isosmotic state, could be interpreted in terms of a change in pore size, as the evidence indicates to be the case in the skin, and as we have inferred to be the case in the frog bladder and the amphibian nephron

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made up entirely of thin limb tissue In man the incidence of long loops is only about one in eight

Sawyer In following what Dr Wirz has said may I mention a fact which is frequently mentioned as evidence against his theory that is the low incidence of long thin loops in man, and the lack of any correlation between the number of long loops and concentrating ability in kidneys from mammals in general His theory does not require however that every nephron has a long loop If only one out of four or six had a long thin loop the hypothetical counter current system would still account for the final concentration of urine in the collecting ducts since the volume of urine that must be concentrated is in the antidiuretic state greatly decreased during passage through the distal convolutions

Wirz I think that the short loops may nevertheless be called loops

Sawyer Yes

de Wardener When the urine is hypertonic in the collecting ducts it is in equilibrium with the thin loops that is why the urine in the collecting ducts is hypertonic Is that right?

Wirz There cannot be much difference

de Wardener And when the urine in the collecting ducts is hypotonic you suggest that the fluid is iso osmotic Is that right?

Wirz My assumption is that in the absence of the antidiuretic hormone the descending limb is virtually impermeable to water Then kidney function would proceed as if there was no counter current system The multiplying action of the counter current depends on the entering of a (slightly) hypertonic solution into the ascending limb

de Wardener My difficulty is that you suggest that there is equilibrium between the thin loop and the collecting tubules only when the kidney is passing hypertonic urine, but this is prevented when the urine is hypotonic

Wirz This is why I have to assume that the antidiuretic hormone influences the water permeability in the collecting ducts as well (see the last figure of my paper)

Sawyer I do not feel that it is necessary to postulate that the hormone alters the permeability of the thin limb or the collecting duct to water If enough water is available the reabsorption of water by the final concentrating mechanism (T_{H_2O}) is continuous under all conditions in which it can be measured (refer to my Fig 5) It is limited by a maximal urine osmotic pressure or by a maximum rate of water reabsorption We have previously assumed that this is an active transport of water and like other active tubular transports it has a maximum rate (T_{mH_2O})

I do not see that it is inconsistent with your theory if we assume that T_{H_2O} is going on continuously to the extent of say 5 per cent of the filtration rate when at least that much free water is delivered to it from the distal tubule We don t have to postulate any changes in T_{H_2O} in diuresis and antidiuresis If in diuresis perhaps 15 per cent of the filtered water is presented at this level and T_{H_2O} reabsorbs 5 per

Discussion

Chairman H Heller

Wirz There is a slight difference in definition between Homer Smith s terminology and that which I have used Smith calls everything after the ending of the thin limb distal What I have punctured is the convoluted part of the distal tubule If we allow for the differences in definition then Dr Sawyer and I are in much greater agreement than might appear

Sawyer I entirely agree I meant to point that out myself Although there is hypo tonic urine in the distal convolutions, it does not rule out the possibility that it is isotonic at the end of the thin limb

Dale I am still a little puzzled Not having followed the modern developments in renal physiology, I find it a little difficult to relate these ideas to my conception of the structure of the nephron When you say distal limb what do you mean? What is the loop of Henle doing? I got the impression that Dr Wirz and Dr Sawyer were using the word distal in different senses

Wirz Next to the glomerulus we have what is commonly referred to as proximal con voluted tubule It consists of a convoluted part and a pars recta which passes down beneath the cortico medullary boundary In the outer zone of the medulla is the beginning of the thin limb which may be long or 'short The thin limb gives way to the thick, ascending limb of the loop of Henle This segment touches the glomerulus of the same nephron, and what I call the distal convolution starts at this point The end of the distal convolution is defined by the branching of the collecting duct This is a somewhat arbitrary definition but it is very convenient as the two end points of my distal convolution are easily recognized in post mortem microdissections All my distal' micropunctures are located between these points Homer Smith would include the thick ascending limb of the loop in what he calls the distal system

Sawyer We use the term distal system to mean anything past the thin limb

Dale Where does the concentrating start?

Wirz I have found by micropuncture the urine in the proximal part of the distal convolutions to be hypotonic in both diuretic and antidiuretic states In the distal parts of the distal convolutions the urine is hypotonic in the diuretic state and iso tonic in the concentrating kidneys So some of the diluting process must occur some where in the ascending limb, and the concentrating process somewhere lower down in the collecting duct

Bargmann Would it be possible that this segment is rather long? Maybe the 'Haupt stuck with brush border reaches the bend

Wirz There are wide species differences among mammals In rats and hamsters there are mostly what I think the anatomists call long loops in which the bend is

Neurohypophysial hormones and the mammary gland

by

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THE phenomenon of lactation can be considered as comprising two main phases first, the phase of milk secretion in which the milk is synthesized in the alveolar cells and passed from the cytoplasm of the cells into the alveolar lumen and secondly the phase of milk removal in which there is a passive component—passive with drawal—and an active component—milk ejection (for terminology see Cowie Folley Cross Harris Jacobsohn & Richardson 1951) These two phases were recognized many years ago by Gaines (1915) who remarked that milk secretion in the sense of the formation of the milk constituents is one thing the ejection of the milk from the gland after it is formed is quite another thing Unfortunately this distinction was overlooked by many investigators in the field of lactational physiology Much confusion thereby arose and the necessity for distinguishing these two phases was stressed by one of us Folley (1947*b*) and again by Cowie *et al* (1951) We now propose to consider the roles which hormones of the neurohypophysis may play in these two phases

MILK REMOVAL

It is convenient to start with a consideration of the second phase since more is at present known of the role of the neurohypophysis in this phase In the lactating mammary gland the greater portion of the milk secreted by the alveolar cells re mains within the alveolar lumen and fine ducts a smaller portion passing into the large ducts and cisterns (or sinuses) This smaller portion can be *immediately* removed by the action of suckling or milking or by cannulation The physiological contractile mechanisms of the gland play no part in the removal of this fraction of the milk and the process may be described as one of *passive withdrawal* The larger portion of the milk in the alveoli and fine ducts must be actively ejected from these positions by the intervention of the special contractile tissue of the mammary gland into the large ducts and cisterns before it becomes available to and can be withdrawn by the suck ling young or the hand milker This process of *milk ejection* is believed to be brought about by the reflex contraction of the myoepithelium (see Plate 1) which forms a network over the stromal surface of the alveoli (Richardson 1949 Linzell 1952) In agricultural circles the milk ejection process is commonly termed the let down of milk The misleading and inappropriate nature of this term was first stressed by one of us some years ago (Folley 1947*b*) and later the term milk ejection was recom mended by a group of workers in this field (Cowie *et al* 1951 and Cowie Folley &

cent that still leaves 10 per cent for excretion That is a fairly high rate of urine flow In the presence of antidiuretic hormone however, the volume of urine delivered from the distal tubule is reduced and $T^c_{H_2O}$ acts to concentrate this smaller volume to an osmotic pressure above that of plasma Our theory presented here is somewhat simpler than yours in that it requires only one site of action for the antidiuretic hormone, namely the distal tubule

Wirz I do not see any serious objection to this I assume an impermeable collecting duct in the absence of antidiuretic hormone because in my experiments ureteral urine is, if anything, more dilute than distal tubular fluid Thus I have some difficulty in visualizing the passage of water in an absolutely fixed amount not dependent upon a concentration gradient

Sawyer I was assuming here that $T^c_{H_2O}$ proceeds continuously with or without antidiuretic hormone There are Berliner s experiments in which he was able to produce a hypertonic urine in a dog in maximal water diuresis If he reduced the filtration rate of one kidney to the correct level, he could get a definitely concentrated urine even though the urine secreted by the other kidney was maximally dilute This seems to be due to the fact that he has reduced the volume of fluid delivered to the concentrating mechanism ($T^c_{H_2O}$) so that this mechanism whatever it may be, is taking out enough water to make the urine hypertonic Shannon, of course, saw the same thing in the diabetes insipidus dog when the GFR was low, but the criticism can always be levelled that the absence of antidiuretic hormone was not complete

Cole I would like to ask Professor Wirz a question about the multiplication principle he has described

In the paper by Hargitay & Kuhn (*Z Elektrochem* 55, 539 1951), there is a diagram suggesting that water goes from the collecting duct into the ascending limb of Henle s loop in Professor Wirz s diagram he suggests that it goes into the descending limb So far as the system is concerned is this of any importance or is the water taken up from the collecting duct primarily removed by the blood vessels

Wirz I think in the first paper of Hargitay & Kuhn in which they describe their artificial model they had just limbs and no interstitial spaces At that time we thought too little of the effect of the blood circulation and only after we found in the golden hamster that the concentration of the blood goes up as high as the concentration of the urine we became aware of the fact that the blood is an integral part of the whole concentrating mechanism Now I think that is not the ascending or the descending limb but mostly the blood which carries the water away



Plate 1

Section of part of the surface of a contracted alveolus from the mammary gland of the goat showing a myoepithelial cell with nucleus (and branching processes (From Richardson 1949)

Richardson, 1954) The term 'milk ejection' was first used by Gaines in his classical paper in 1915 and we still consider it to be the best yet brought forward or invented for use in scientific literature to describe this process

MILK EJECTION REFLEX

Neuro hormonal arc

The reflex contraction of the myoepithelial cells is normally brought about by the stimulus of suckling or milking Like other reflexes, this milk ejection reflex can be conditioned In the milking shed the cow readily becomes conditioned to a variety of auditory tactile and visual stimuli associated with the act of milking such

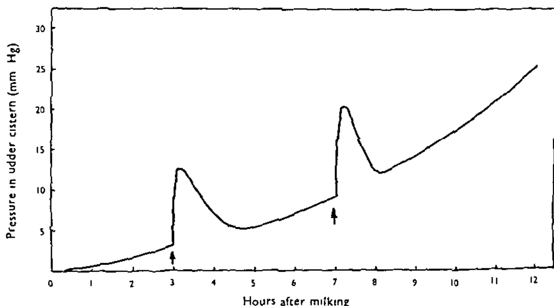


Figure 1

Milk pressure curve (cow) between one milking and the next showing the reaction to the milking stimulus on milk however being withdrawn Arrows indicate application of stimulus (Redrawn from *T et el* 1926)

as the rattle of milk buckets washing of the udder and appearance of the milking machines It can also be conditioned experimentally to the ringing of a bell (Gracey 1952) Waller (1938) in his book has described similar examples of conditioning in lactating women The milk ejection reflex manifests itself by a sudden rise in the milk pressure in the mammary gland This rise in pressure is well illustrated in Fig 1 taken from a paper by Tgetgel (1926) which shows the curve of increase of milk pressure in the mammary cistern of a cow between one milking and the next Two successive applications of the milking stimulus at times marked by the arrows produced a *sudden increase in the pressure of the milk within the cistern which soon* subsided even though no milk was withdrawn The milk ejection reflex is thus the means by which the active but unconscious participation of the lactating mother comes into play so that the full yield of milk can be obtained by the suckling young

or by the milker Even after normal milk ejection however, the gland cannot be completely milked out and there is always a small quantity of milk left in the gland This residual milk can only be obtained if oxytocin be injected

The milk ejection reflex was once believed to consist of a purely neural arc but as we shall see there is now much evidence for the view that the arc is a neuro hormonal one the efferent component of which is a hormone in all probability oxytocin released from the neurohypophysis into the blood stream On reaching the mammary gland this hormone is believed to evoke the contraction of the myoepithel ium

It is over forty years since Ott & Scott (1911) and Schafer (1913) observed that the milk ejection reflex could be mimicked by the injection of extracts of the posterior lobe of the pituitary Gaines (1915) carried out a series of experiments on the milk ejection reflex and he showed *inter alia* that anaesthesia abolished the reflex in the nursing bitch and that this inhibition could be overcome by the injection of posterior pituitary extract (Fig 2) Despite his clear cut results which today would be inter

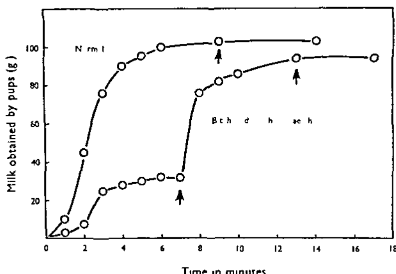


Figure 2

Milk flow curves from a bitch under normal conditions and under the anaesthesia Arrows indicate injection of posterior pituitary extract (Redrawn from Gaines 1915)

preted as strongly suggesting a physiological role for the neurohypophysis in milk ejection Gaines regarded the milk ejection reflex as a neural arc and the action of posterior pituitary extract *as only of pharmacological interest* It was not until fifteen years later that Turner & Slaughter (1930) suggested that the milk ejection reflex might act through the posterior pituitary gland It was another eleven years before this possibility was further explored and its practical significance realized Ely & Petersen (1941) showed that milk ejection could occur in response to the normal milking stimulus in the half of the bovine udder in which the two nerves



a



b

Plate II

Abdominal mammary glands of rats from which the pups were removed at the 4th day of lactation the mother thereafter receiving intraperitoneally injections of (*a*) 1.0 ml saline thrice daily for 9 days and (*b*) 1.0 I.U oxytocin in 1.0 ml saline thrice daily for 9 days

TABLE I

Amounts of oxytocin and vasopressin in posterior pituitaries of goats
(Unpublished work of Margaret H I Dodd cited by Folley 1952*a*)

No of goat	Sex	Age	Vasopressin I U /mg dry tissue	Oxytocin I U /mg dry tissue
		Non lactating goats		
393	♀	6 months	0.7–0.8	0.8
385	♀	8	1.8	1.8
342	♂	2½ years	0.5	0.6
		Lactating goats unmilked for 24 hr before autopsy		
316	♀	2 years	0.6	0.8
326	♀	?	0.6	0.6
338	♀	2	0.8	≃0.7
100	♀	6½	0.8	0.8
		Lactating goats killed immediately after milking out		
79	♀	8 years	0.7	0.65
127	♀	6	≃0.6	≃1.0
165	♀	5	0.8	0.8–1.0
291	♀	3	0.6	0.8
232	♀	4	0.8	0.8–1.0
222	♀	4	0.6	1.0
226	♀	4	0.28 (?)	0.7

given results which are at present difficult to evaluate Stutinsky (1953) reported that twenty four hours after parturition in the rat there was a dramatic loss of neuro secretory material from the neurohypophysis which did not occur if suckling was prevented Malandra (1955) also reported a reduction of neurosecretory material in the neurohypophysis of the rat during early lactation Other workers have ob served a loss of neurosecretory material in early lactation in the guinea pig (Collin & Racadot, 1953) and in the mouse (Brightman 1955) On the other hand Drager & Rennels (1955) could find no consistent alteration in the amount of neurosecretory material in the rat neurohypophysis associated with parturition or lactation While studies on the effect of the milking or suckling stimulus on the amount of hormonal or neurosecretory material in the neurohypophysis have added no convincing evid ence in support of the neurohypophysial participation in the milk ejection reflex it is equally true that they provide no evidence against such a view

Antidiuresis

Indications have been obtained by another procedure that the milk ejection reflex involves activation of the neurohypophysis The occurrence of an antidiuretic effect of the posterior pituitary type shortly after the application of the suckling or milking

believed to carry all the efferent fibres to the gland, had been cut Evidence was also obtained that both the reflex and the action of injected oxytocin could be inhibited by the injection of adrenaline thus providing an explanation for the well known observation that fear or pain may inhibit the normal milk ejection reflex It was further shown that blood collected from cows after stimulation produced ejection of milk when perfused through the isolated cow udder (Petersen & Ludwick, 1942) Petersen and his colleagues concluded that the milk ejection reflex embodied a neurohormonal arc and that the reflex could be initiated by the suckling or milking stimulus or by stimuli arising from the sight or sound of events which the cow had come to associate with the beginning of milking It was further postulated that once oxytocin was liberated into the blood stream it was quickly inactivated and hence rapid milking was desirable to obtain proper emptying of the gland This work of Petersen aroused wide interest because of its physiological and practical importance Despite the attractiveness of his postulates, however the experimental evidence on which they were based was scanty and our colleague Mrs M H I Macaulay (1951) reviewing the literature some ten years later concluded that there was insufficient evidence to do any more than suggest that the neural lobe participates in milk ejection In the last five years however more evidence has accumulated to confirm the role of the neurohypophysis in milk ejection and we shall now briefly consider this evidence

Hormone content of neural lobe

Attempts to demonstrate alterations in the hormone content of the neural lobe following the suckling or milking stimulus have led to no clear conclusions Mrs M H I Dodd (formerly Mrs Macaulay) in our laboratory, compared the oxytocic and pressor hormone contents of the neural lobe of goats killed before milking with those of the neural lobe of goats killed immediately after milking (Table 1) but observed no significant depletion of either hormone (see Folley 1952*a*) Subsequent experiments both in our laboratory and by Denamur & Martinet (1953) suggested that the quantity of oxytocin necessary for evoking milk ejection in the goat was only 10 per cent or perhaps much less of the total hormone content of the gland and it is therefore hardly surprising that no significant depletion was observed since the small quantity of oxytocin released may have been well within the limits of error of the assay procedure Similar experiments in the cow by Whittlestone, Bassett and Turner (1952) likewise failed to show any appreciable depletion In some smaller species however (the dog, cat guinea pig and rat) a pronounced decrease in oxytocic activity of the pituitary has been observed during lactation by Dicker & Tyler (1953) This observation has been confirmed in the case of the dog by van Dyke Adamsons and Engel (1955) and in the rat by Professor Fromageot as he has reported to us in his paper to this symposium It is possible that the differences may represent species variation in the amount of oxytocin necessary to produce milk ejection or in its replacement rate following liberation

Neurosecretory material

Histochemical studies on the variations in amount of the neurosecretory material present in the hypothalamus and neurohypophysis during lactation have likewise

electrical stimulation of the hypothalamus and interruption of nerve tracts within the hypothalamus by electrolytic lesions Cross & Harris (1950, 1951 1952) showed that electrical stimulation of the supraoptico hypophysial tract caused milk ejection in the anaesthetized rabbit Rabbits, moreover with lesions in this tract failed to exhibit milk ejection on electrical stimulation of the tract central to the lesion and the litters of these rabbits were unable to obtain significant quantities of milk on suckling unless the mother was injected with oxytocin Andersson (1951*a*, *b*) evoked milk ejection in lactating ewes and goats by electrical stimulation of hypothalamic centres in or adjacent to the supraoptic nucleus Andersson concluded that these responses were undoubtedly hormonally mediated because they could be elicited during sacral anaesthesia or in denervated udder halves He further showed that blood taken from a goat immediately after electrical stimulation evoked a prompt flow of milk from the cannulated teats of another lactating animal on intravenous injection thus demonstrating that the stimulation had caused the discharge of a milk ejection hormone into the blood stream

Posterior lobectomy

Experiments on the effect of removal of the neural lobe on the milk ejection reflex have given results which are at first sight confusing Smith (1932) in a study on the effect of ablation of the posterior lobe on parturition in the rat observed that rats so treated could raise their young and he concluded that removal of the posterior lobe did not seriously affect lactation Houssay (1935) also claimed that bitches lactated normally after removal of the posterior lobe On the other hand Harris & Jacobsohn (1952) in the course of studies on the transplantation of the pituitary in the rat observed that hypophysectomized rats possessing functional anterior pituitary grafts could only rear their young if regularly injected with posterior pituitary extract In agreement with this are the observations of Cowie (quoted by Folley 1952*a*) that when the posterior pituitary is removed from rats on the fourth day of lactation such rats are unable to raise their litters unless injected two or three times daily with oxytocin An explanation of these contradictory findings on the effect of posterior lobectomy may be forthcoming from some recent experiments by Benson & Cowie (1956) who have confirmed that the milk ejection reflex is abolished in the rat after posterior lobectomy but that if such rats again become pregnant they then rear their second litter without requiring injections of oxytocin (Fig 3) In the rat at least it would appear that on removal of the neural lobe other parts of the neurohypophysis —the neural stalk or the median eminence—can take over the function of the posterior lobe in the milk ejection reflex Benson & Cowie by measuring the volume of the neurohypophysial tissue from serial sections have demonstrated that after posterior lobectomy the neural stalk hypertrophies (Table II) This is in agreement with the findings of Stutinsky (1952) and Billenstien & Leveque (1955) who report that after hypophysectomy there is an hypertrophy and reorganization of the end of the stalk which gradually assumes the appearance of a small neural lobe It is of interest to note that whereas there was at least a partial restoration of the milk ejection reflex the rats continued to show diabetes insipidus (Table III) Further experiments are in progress to decide how long after removal of the neural lobe it is before the milk ejection reflex reappears Since Smith (1932) lobectomized his rats

stimulus has been demonstrated in the water loaded animal Such results have been obtained in the rabbit by Cross, (1950) the cow by Peeters and Coussens, (1950), the woman by Kalliala and Karvonen, (1951) and the bitch by Kalliala, Karvonen and Leppanen (1952)

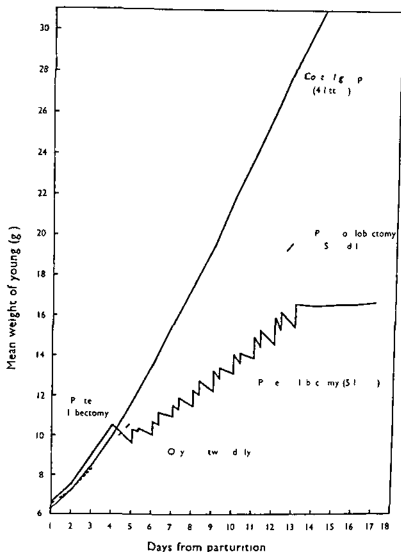


Figure 3

Growth curves of litters of (*a*) control rats (4) (*b*) rats (5) lobectomiz d on fourth day after parturition and receiving oxytocin (0 75 I U) intraper toneally twice daily from day 5 to day 13 (*c*) same lobectomized rats in second lactat on but receiving no oxytocin

Hypothalamic stimulation and lesions

The most convincing evidence however of the intervention of a hormone of the neurohypophysis in the milk ejection reflex comes from experiments involving

the posterior lobe possess milk ejection activity the oxytocic fraction is much more active in this respect than the pressor fraction (for references see Folley 1954) However, the activity of the pressor fraction is considerably more than can be accounted for on the basis of contamination with the oxytocic factor The earlier experimental evidence relating to this question was discussed by Folley (1954) who concluded that in view of the uncertainties associated with the present assay procedures for oxytocin it seemed reasonable on the basis of the available evidence to believe that milk ejection and oxytocic activities are facets of the same biological activity characteristic of the oxytocic polypeptide and that both activities are also possessed in some small degree by the vasopressor polypeptide Studies on the highly purified or synthetic polypeptides of du Vigneaud have now confirmed this view (for references see review by van Dyke Adamsons & Engel 1955) and the discrepancies arising from different assay procedures are well illustrated by the recently published data of van Dyke, Adamsons and Engel (1955) which show that when purified *oxytocin* is assayed by three different procedures (rat uterine avian depressor and milk ejection methods) the potency is the same in all cases but when purified *vasopressin* is assayed by these methods the potency as determined by the avian depressor and milk ejection methods is about three times that obtained by the rat uterine method

Inhibition of milk ejection reflex

Of great scientific and practical interest are the experiments on the various physiological and psychological factors which may interfere with or inhibit, the normal milk ejection reflex It has long been known to the farmer that fright or discomfort can inhibit the milk ejection reflex in the cow (Ely & Petersen 1941) Clinicians have made similar observations regarding the nursing mother (Newton & Newton, 1948) As previously stated the possibility that sympathetic activity might play a part in the phenomenon was suggested by Ely & Petersen (1941) who found that the injection of adrenaline interfered with milk ejection in the cow They believed that the suppression was peripheral in nature and occurred in the udder Inhibition of milk ejection by injection of adrenaline has been reported in the sow by Braude & Mitchell (1952) and in the rabbit by Cross (1953) The nature of this inhibitory process has been extensively studied by Cross (1953 1955*a*) who showed that in the anaesthetized rabbit stimulation of the posterior hypothalamus inhibits milk ejection in a manner similar to that of physiological doses of exogenous adrenaline He showed moreover that adrenalectomy abolishes the inhibitory effect of posterior hypothalamic stimulation Cross was of the opinion that constriction of the mammary blood vessels was the major factor involved in this peripheral block of the mammary response to circulating oxytocin and recent studies by Linzell (1955) on the action on the myoepithelium of topically applied drugs support this view While the experiments of Cross clearly showed that central stimulation by causing sympathetico adrenal activity could inhibit the milk ejection response to oxytocin Cross was careful to point out that it did not necessarily follow that this mechanism was the one normally in action in the conscious animal suckled under conditions of emotional disturbance In a further series of experiments Cross (1955*b*) showed that the main factor in emotional inhibition of milk ejection in the conscious rabbit was a central block of

some weeks before they became pregnant it is likely that the reflex was at least partly functional by the time suckling started

TABLE II

Volume of residual hypophysial tissue in the rat after hypophysectomy and posterior lobectomy
(From Benson & Cowie 1956)

Treatments	Volume (mm ^{3}) of		
	Anterior lobe	*Intermediate lobe*	*Neurohypophysis*
Control rats (7)	3 21 ± 0 23	0 16 ± 0 01	0 36 ± 0 01 (stalk + median eminence = 0 10 ± 0 01)
Posterior lobectomy killed immediately after operation (6)	2 33 ± 0 31	—	0 09 ± 0 01
Hypophysectomy killed immediately or within 14 days of operation (13)	—	—	0 09 ± 0 01
Posterior lobectomy killed 4–5 months after operation (14)	2 46 ± 0 22	(0 027 in one rat only)	0 15 ± 0 01

It is apparent from what has been said that the evidence for the neuro endocrine regulation of milk ejection by a pathway involving activation of the neurohypophysis is impressive in amount and in sum convincing

TABLE III

Effect of posterior lobectomy on urine production in the rat
(From Benson & Cowie 1956)

Treatments	Mean urine volume (ml) over 48 hr period		
	After 1st lactation	After 2nd lactation	Before killing
Control rats (5)	27 ± 2 5	22 ± 2 2	20 ± 2 0
Posterior lobectomy (17)	54 ± 4 9	49 ± 4 5	38 ± 4 0

The nature of the milk ejection hormone

It is now necessary to consider briefly the nature of the milk ejection hormone It has uniformly been found that while both the oxytocic and vasopressor fractions from

who suggest that the chemotransmitter for the release of gonadotrophins may be carried in the neurosecretory material associated with the hypothalamic nuclei and hypothalamo hypophysial tract, thus implying an integration of anterior and posterior lobe functions In this connexion there have been in the past few years several studies on the possible control of adrenocorticotrophin release by posterior pituitary hormones (Fraja & Martini 1952 Mirsky Stein & Paulish, 1954, Barrnett 1954 Scharrer & Scharrer 1954 Shibusawa Saito Fukuda, Kawai & Yoshimura 1955 and Martini & Morpurgo, 1955)

Neurohypophysis and control of secretion of lactogenic and galactopoietic hormones

The fact that the suckling stimulus as we have seen appears to play a central role in the two main phases of lactation—milk secretion and milk ejection led us to speculate whether the reflex release of oxytocin might not provide a hormonal link between these two phases This concept that the release of the lactogenic and galactopoietic factors from the anterior lobe may be controlled by oxytocin is an intriguing one since it would provide a simple explanation of how the hormonal integration and co ordination of mammary function is achieved The oxytocin liberated as a result of the suckling or milking stimulus would thus not only cause contraction of the myoepithelium and ejection of milk from the alveoli, but would liberate the lactogenic and galactopoietic factor(s) from the anterior lobe which are necessary for the maintenance of further secretion of milk by the alveolar cells

In order to test the validity of this concept Mr Benson in our laboratory has been carrying out some preliminary experiments which have so far given encouraging results (Benson & Folley 1956) One approach to this problem has been to investigate whether the rapid involution of the mammae which occurs after weaning can be prevented by frequent injections of oxytocin It will be recalled that Williams (1945) investigating Selye s theory has demonstrated that these involutionary changes could be retarded by the injection of prolactin just as they could by suckling and it should therefore be possible to get a similar retardation with oxytocin if oxytocin can release prolactin In Mr Benson s experiments the pups were removed from 44 lactating rats on the fourth day of lactation Eight of these rats were kept as controls and received daily injections of 1 ml saline intraperitoneally three times a day The remainder were divided into three groups of 12 and given respectively 0 25 1 0 and 1 5 I U oxytocin intraperitoneally three times a day Four of the animals in each of the oxytocin treated groups were killed after 3 6 and 9 days of treatment Four controls were killed after 6 and 9 days of treatment Histological sections of the left abdominal gland were prepared and classified subjectively according to the degree of glandular involution which had occurred and also by a quantitative procedure based on measuring the mean diameter of the alveoli No differences were detected between the control glands 6 days after weaning and those receiving oxytocin for 3 and 6 days Involution was markedly retarded however in the glands of the animals receiving oxytocin for 9 days in contrast to glands of the 9 day control animals Sections of glands of the oxytocin treated animals showed mammary alveoli distended with milk whereas in the control glands there were only scattered islands of small disintegrating alveoli which in many places had become reduced to clusters of epithelial cells (Plate II) In agreement with these subjective observations the mean

release of oxytocin since the injection of oxytocin could restore normal milk removal during emotional disturbance Only in a few cases was this unsuccessful, indicating a concomitant activation of the sympathetico adrenal system These experiments of Cross, moreover, provide further confirmation of the essential role of oxytocin in milk ejection As Dr Cross is here with us he will no doubt wish to say something about his work and we will not therefore deal further with it

MILK SECRETION

So far we have been considering only one phase of lactation namely milk removal The other phase that of milk secretion, is a process known to be under the hormonal control of the anterior pituitary We need not here go into the debatable question whether there is one lactogenic factor—prolactin or a complex of factors of which prolactin is only a component (for references, see reviews by Folley, 1952*a* *b*) but we now wish to consider briefly whether there may not be an integration of the functioning of the two main phases of lactation by means of the hormone(s) of the posterior lobe

It has been recognized for some years that the suckling or milking stimulus not only results in the reflex ejection of milk but also results in the reflex liberation of the lactogenic and galactopoietic factor or factors from the anterior lobe It may be recalled that the rapid involution of the mammary gland following weaning of the young was once believed to be due to the effects—perhaps chemical, perhaps physical —of accumulation of milk within the alveoli The observations of Selye (1934) first provided an indication that the removal of the suckling stimulus was a more important factor than the non removal of the secretion in causing the onset of mammary involution after weaning Selye showed, *inter alia*, that when the main milk ducts to the nipples of the lactating rat were ligated so that milk could not be removed from the glands, rapid involution of the gland such as occurred in non suckled animals did not occur if suckling were continued Selye and his collaborators suggested that the suckling stimulus initiated a nervous reflex which elicited the secretion of prolactin from the anterior pituitary which maintained the functional activity and structural integrity of the gland These experiments of Selye have since been confirmed and extended by several investigators (for references see review by Folley 1947*b*)

The question therefore arises as to what extent the arcs of these two reflexes elicited by the suckling or milking stimulus share common paths Little appears to be known about the afferent neural paths but it is not improbable that a common path is shared as far as the hypothalamus Thereafter the route to the posterior lobe is likely to be *via* the hypothalamic nuclei and hypothalamo hypophysial tracts There is much less certainty, however about the route followed by stimuli passing from the hypothalamus to the anterior lobe Harris (1955) considers that there is convincing evidence in favour of the view that the link is a neurohormonal one achieved by the liberation from nerve fibres from the hypothalamus of some humoral substance(s) or chemotransmitter into the primary plexus of the hypophysial portal system It is believed that this substance is then carried in the portal circulation to the anterior lobe Various suggestions have been made regarding the nature of the chemotransmitter substance Some authorities however question its reality (Zuckerman, 1954) One view of particular interest to us here is that of Benoit & Assenmacher (1953)

the hormone with blood from other parts of the body had occurred Landsmeer (1951) has shown that such direct vascular connexions from posterior to anterior lobe are present in the rat Daniel & Prichard (1956) have confirmed and extended Landsmeer s observations in the rat and are now investigating the vascular connexions between the pituitary lobes in the sheep (Daniel private communication) With reference to the question of vascular connexions between the lobes of the pituitary, it may be noted that if the anterior lobe receives blood rich in oxytocin direct from the posterior lobe then in experimental studies it may well be necessary to inject apparently unphysiological quantities of oxytocin into the general circulation in order to achieve the physiological levels necessary for stimulation of the adenohypophysis

In the past as its name implies the main physiological action attributed to oxytocin has been its effect in stimulating uterine contractions at parturition although Harris (1955) has recently pointed out that unequivocal proof of this role is still lacking We can, however, now claim with some conviction that oxytocin has another important and even better established physiological function namely its role in milk ejection There is moreover as we have discussed the further possibility that oxytocin may be responsible for the release of the anterior pituitary factors necessary for milk secretion If this be so, then it becomes the all important hormonal factor responsible for the integration and co ordination of mammary function This possibility is an attractive one It has both the merit of simplicity and as we have shown is capable of explaining many observations but further investigation is required before it can be accepted as fact

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alveolar diameter was significantly greater in the oxytocin treated animals ($P < 0.01$) While it does not necessarily follow from these experiments that oxytocin is the normal stimulus to the anterior pituitary causing the release of the galactopoietic factor(s) under physiological conditions it is clear that this possible role of oxytocin requires further investigation We consider it unlikely that these results are due to a direct effect of oxytocin on the mammary gland because Dr T R Bradley in our laboratory has found that prolactin alone of a number of anterior and posterior pituitary principles tested, including oxytocin, had a localized lactogenic effect when injected intraductally into the mammae of suitably prepared rabbits Experiments are being planned, however, to study the effects of oxytocin in rats hypophysectomized at the time of weaning

If the above views on the role of oxytocin be correct it is reasonable to believe that under certain circumstances the repeated injection of oxytocin into a lactating animal might result in an increase in milk production by virtue of the release of extra amounts of the galactopoietic factors from the anterior pituitary In this connexion it is most necessary to distinguish clearly between apparent and true increases in milk yield It has long been established that after a normal milk ejection there remains in the udder a quantity of residual milk which can be obtained if oxytocin be injected intravenously This is not of course a galactopoietic effect since the extra milk so obtained will be compensated for by a reduced yield at the next milking unless oxytocin be again injected (for references see review by Folley, 1947*a*) On the other hand there is evidence that when oxytocin is injected daily over periods of time a true increase in yield is obtained In 1944 Knodt & Petersen reported that the rate of decline of the milk yield in cows could be diminished if the residual milk was removed by injections of oxytocin after each milking This effect has in the past been ascribed to the beneficial effects on the functions of the secretory cells of more efficient removal of milk from the alveoli thus preventing the build up of intra alveolar pressure Recent experiments by H G Turner (1955) and Elliott & Brumby (1955) however, cast some doubt on this explanation since these workers have shown independently that the increase in intra alveolar pressure does not affect the rate of secretion until some twenty hours after milking Since the cows in the experiment of Knodt & Petersen were milked at twelve hour intervals the galactopoietic effects they observed may well have been due to the exogenous oxytocin rather than to the beneficial effect on alveolar pressure of removal of the residual milk Increases both in milk and butter fat yield of cows following regular injections of oxytocin have been reported by Adams and Allen (1952) Sprain Smith Tyler & Fosgate (1954), and Donker, Koshi & Petersen (1954) It is also of interest to note that Donker, *et al* (1954) considered that the increases in yield observed by them were sufficiently large to indicate that some factor in addition to removal of residual milk must be playing a stimulatory role, and that as early as 1944 Petersen tentatively suggested that oxytocin might stimulate the release of lactogenic factors from the anterior pituitary

With reference to the above views on the release of the lactogenic and galactopoietic factors from the anterior lobe it is necessary to consider the nature of the vascular connexions between the two lobes If there were a direct flow of blood from the posterior lobe to the anterior lobe then conditions would be ideal as the anterior lobe would then receive blood highly charged with oxytocin before any dilution of

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Zuckerman The histological appearance of the tuber cinereum of the ferret is different from that of the neural process What I am concerned with is the belief—and Dr Pickford knows more about this than I do—that the neural process itself does some thing in the sense of a chemical transformation to whatever hormone may be stream ing down from the hypothalamus The actual structure of the neural process is always different from the median eminence both before and after stalk section Does differentiation in the morphological sense really occur in the central stump of the stalk and transform it into a neural process capable of transforming the presumed hypothalamic secretion?

Pickford The impairment of milk ejection really does parallel experimental diabetes insipidus, for if you do an incomplete section of the tract or if you remove only the posterior lobe and part of the stalk you then get polyuria for about 10–14 days followed by recovery to a normal water output Perhaps in the dog the time relation ships may not be the same as in the rat, but the general idea is the same Why this temporary change should occur we do not know it may be a clot

I wish to ask Dr Folley if the second lactation enhances the apparent hypertrophy, or does this occur in the absence of a second pregnancy and lactation?

The effect of oxytocin on the release of prolactin is one thing Dr Brooks and I must naturally take into account in relation to sodium excretion by the kidney Does it mean that oxytocin is doing something to the anterior lobe and thus indirectly to the suprarenal cortex?

Cowie With regard to the question of the possible effects of the second lactation we have not examined serial sections from animals after the first pregnancy but I believe Dr Stutinsky has relevant observations

Stutinsky I was extremely interested by the findings of Dr Folley and I was glad to hear of his results which confirm my work

I should like to show a slide which shows a horizontal section of the hypothalamus from a rat neurohypophysectomized two months before A part of the anterior lobe is present there is no pars intermedia and the end of the stalk has enlarged This enlarged end is reorganized and shows a normal neural lobe structure which reacted to hypertonic saline in a normal manner

Another slide shows the structure of the neural lobe of a normal rat during labour This lobe contains much neurosecretory substance In the following slides you can see the depletion of this material which occurs twenty four hours after parturition

Zuckerman Is it a constant finding that a neural process as such is redifferentiated after the definitive one is removed?

Stutinsky In the rat always

Zuckerman In at least two other species the ferret and monkey nothing like this happens Once the neural process is destroyed it does not reform

G W Harris I am disturbed by one aspect of regeneration of the neural process There is a great deal of evidence that the cells of the supraoptic and paraventricular nuclei largely atrophy and disappear after sectioning the supraoptico hypophysial

Discussion

Chairman Sir S Zuckerman

Zuckerman Dr Folley will of course be aware that myoepithelial cells were first described other than in the mammary gland Presumably the responses of the mammary myoepithelial cells to oxytocin, described by Folley and Cowie, are not associated with corresponding responses by similar cells in other secretory tissue, for instance, the salivary glands

When speaking of the rate of growth of young after posterior lobectomy of the mother, Dr Folley implied that certain changes had occurred in the stalk which re created from what was left of the neurohypophysial tissue (median eminence and the other parts of the stalk) a new neural process, and in this connexion he referred to the work of Stutinsky and others What does he really think happens to the remaining tissue? Is there any reason why neurosecretory substances should not be emerging *from the central part of the stalk immediately after lobectomy? Or is he suggesting* that after the operation there is some kind of differentiation of the central stump of the stalk which results in the re establishment of the same kind of cellular structure which we find in the neural process?

Cowie We have not been able to investigate this yet We merely draw attention to the fact that after posterior lobectomy milk ejection in response to suckling does re turn and at the same time there is an hypertrophy of the end of the stalk There may not be any direct connexion between the two things

Zuckerman Is it conceivable that the failure of the young to grow after lobectomy of the mother, is related to the operation itself?

Cowie Sham operations in which the hypophysis is exposed but not removed have no effect on the growth of the young Further, in a few experiments, I accidentally removed only half the posterior lobe and in these cases the growth curve was in hibited only for part of the first lactation

G W Harris We have observed the tuber cinereum not after posterior hypophy sectomy but after stalk section and after total hypophysectomy in rats, rabbits and ferrets We have found that the stalk remnant and median eminence show thrombosis in the first twenty four hours after operation Together they appear as a small hard nodule, cherry red in colour which is in contrast to the white hypothalamus This is presumably due to the blockage in blood flow in the hypophysial portal vessels At a later date the vessels seem to recanalize and the primary plexus of the portal vessels is reformed Recent observations of Dr B T Donovan and Dr J J van der Werff ten Bosch (*Proceedings of Physiological Society* 20–21 April 1956) show that after pituitary stalk section in the ferret the median eminence appears as a globular mass instead of as a flat plate when viewed in sagittal section They have not yet made measurements of the tissue volume of the tuber cinereum in the ferret but simple inspection of the slides gives the impression that the tuber cinereum has increased in mass I wonder if these observations might explain the temporary inhibition of milk ejection described by Dr Cowie and Dr Folley

Bargmann May I add a detail about the connexion between the neurosecretory system and the adenohypophysis, they are connected by special vessels in the infundibular wall, vessels which are surrounded by thick and swollen fibres containing neurosecretory substance This may be the site of liberation of hormone into the blood vessels

Martini In connexion with Dr Folley s observation that oxytocic hormone seems to stimulate the release of lactogenic hormone we (Martini & Morpurgo, *Nature*, (*Lond*) **175**, 1127 1955, Martini & de Poli, *J Endocr* **13**, 229 1956) have shown, that both the antidiuretic and the oxytocic hormone may produce the release of corticotrophin and thyrotrophic hormone These experiments appear to indicate that antidiuretic hormone and oxytocin may act as neurohormonal agents linking the hypothalamus to the adenohypophysis

Folley With regard to Dr Pickford s remarks about the rats in the second lactation in which the milk ejection reflex had returned at least for the greater part, it should be noted that the rats still exhibited polyuria

Cowie I certainly agree with Dr Chester Jones about the difficulty of neurohypophysectomy in the rat and because of this we made careful measurements of the volume of neurohypophysial tissue present both immediately after lobectomy and several months later We found a significant increase in volume We did not measure water intake but immediately after the first lactation the lobectomized rats were excreting twice the volume of urine excreted by the controls They were still excreting twice the volume after the second lactation

tract There are several people in this room, for example, who have published figures showing a greatly reduced number of cells in these nuclei after hypophy sectomy It is difficult to see, therefore, if the hormone is originating in these nuclei, how 'regeneration at a lower level in the system, that is at the end of the stalk, could increase the discharge of neurohypophysial hormone It would seem better to replace the term 'regeneration by 'reorganization It certainly seems possible that the thrombosed region of the tuber cinereum and pituitary stalk, following stalk section or hypophysectomy can become reorganized in the sense that the vessels are re canalized and the region once more takes over the function of discharging posterior pituitary hormones into the blood stream

Sloper I wish to make a plea for the more accurate use of the word 'hypertrophy, for from what Professor Harris has said about the hypophysectomized ferret and from my experiences with the hypophysectomized human, I suspect that the swellings noted by Dr Cowie and Folley could have been due to infarction and oedema processes which in themselves could impede for a time the release of hormone from neurosecretory fibres in the median eminence Certainly in the human, both thirty two hours and ten days after hypophysectomy there were severe infarcts which ascended the pituitary stalk and spread well up into the median eminence In the human unlike the rat, the accumulation of neurosecretory material in this region does not persist and no regeneration of the type described by Stutinsky develops In fact, I think we may have been attributing too much importance to this question of neurohypophysial regeneration by which I mean the persistent accumulation of vascularized neurosecretory tissue in the median eminence For in the human 100 and 240 days after hypophysectomy no such change occurs On the other hand some neurosecretory material can be found in the median eminence presumably derived largely from the caudal half of the paraventricular nucleus, where about half the normal number of large neurones survive, many of them containing neuro secretory material Since these patients did not have overt diabetes insipidus, in spite of treatment with cortisone, it is probable that they retained a fair measure of neuro hypophysial function I believe therefore that we must attach importance not so much to the regeneration of a neurohypophysis but rather to the persistence of some intact median eminence and the survival of cells capable of neurosecretion

Zuckerman With reference to the duration of the effect I can report that in a series of monkeys subjected to stalk section diabetes insipidus ensued and in some cases con tinued for a year or more

Chester Jones In the rats the effects of neurohypophysectomy vary considerably The best results are obtained by removal of the posterior lobe taking it as high up the stalk as possible, together with minimal disturbance of the anterior lobe It is possible to produce permanent diabetes insipidus in some rats with from four to five times the normal intake of water and with attenuation of the supraoptic and para ventricular nuclei, in such cases I have not seen re growth of the neural lobe Water intake may be used as functional criteria of the lesion produced It may be legitimate to assume that in rats showing no obvious antidiuretic hormonal effect oxytocin secretion is also minimal

Bargmann May I add a detail about the connexion between the neurosecretory system and the adenohypophysis, they are connected by special vessels in the infundibular wall, vessels which are surrounded by thick and swollen fibres containing neurosecretory substance This may be the site of liberation of hormone into the blood vessels

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On oxytocin and uterine function

by

R J FITZPATRICK

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SINCE it was first demonstrated fifty years ago by Dale that posterior pituitary extracts have the property of inducing contractions of uterine muscle, our understanding of this relationship has lagged behind corresponding advances linking the neurohypo physis with kidney and mammary function To a large extent this may be attributed to the intermittent nature of myometrial activity especially its brief activation at parturition and about the time of mating which has rendered quantitative investiga tion difficult These difficulties are enhanced by our lack of knowledge of the physio logical criteria by which we should measure the functional activity of the uterus as an organ, this gives rise to obvious difficulties in interpreting for instance the nor mality or otherwise of parturition in animals or in women with lesions of the neuro hypophysial system (Fisher Magoun & Ranson 1938 Harris 1955 and others)

Newton (1937) focused attention on this problem when he stated that Mere contraction of the uterus is too primitive a process to be dignified by the name of parturition , and he presented evidence subsequently extended by other workers (Bonnycastle & Ferguson 1941 Adler Bell & Knox, 1944 Schild, Fitzpatrick & Nixon 1951) that activation of the myometrium at parturition follows a co ordinated pattern for different parts of the uterus This is discussed more fully below in relation to the nature of uterine response to oxytocin but before embarking on this it is appropriate to consider briefly the evidence suggesting that myometrial function is probably under neurohypophysial control

Two main functions have been ascribed to oxytocin in relation to the control of uterine contractility

(*a*) In the non pregnant female at the time of mating to propel seminal fluid in a rostral direction

(*b*) In the pregnant female at term to propel the foetus(es) in a caudal direction

Release of oxytocin in relation to seminal transport

Rothschild (1953) has indicated on the basis of the purely physical study of sperm atozoan movement that such movement is essentially random in nature and that spermatozoa deposited in the female reproductive tract cannot progress towards the fallopian tubes unless they are impelled by some external propulsive force such as uterine contraction

This is in keeping with the demonstration in many species that the ascent of sperm atozoa in the female tract occurs too rapidly to be accounted for by the flagellate motility of the spermatozoa (in the rat—Hartman & Ball 1930 Blandau 1945 the dog—Evans 1933 the rabbit—Krehbiel & Carstens 1939 the guinea pig—Florey & Walton 1932 the cow—VanDemark & Moeller 1951 the sheep Starke 1949)

Not only is ascent too rapid to be accounted for by independent spermatozoan

movement but moreover, this phenomenon is seen with non motile spermatozoa (VanDemark & Moeller, 1951) Rapid ascent of semen has been demonstrated in the absence of the male (Krehbiel & Carstens, 1939, VanDemark & Moeller, 1951) which eliminates copulation itself as an essential propulsive force, further it is seen with fluids other than semen (Krehbiel & Carstens, 1939, Rowson, 1955) indicating that the pharmacologically active substances known to be present in semen (von Euler, 1937) are non essential

It is reasonable to conclude that the explanation of this rapid transport lies pre dominantly, or entirely with the female and this has led to the view that sperm propulsion after natural mating is effected by uterine contractions—possibly due to the release of oxytocin Oxytocin has in fact been stated to increase the rate of ascent of spermatozoa and other fluids in the isolated perfused bovine uterus (Hays & VanDemark 1952) and *in the intact cow (Rowson 1955)*

An increase in contractile activity of the uterus compatible with this hypothesis has been demonstrated after natural mating, after tactile stimulation of the external genitalia and after mechanical stimulation of uterus or cervix, as indicated in Table 1 It is possible that uterine contraction after stimuli such as these may be explained in terms of local organ response or very simple nervous reflexes However Ferguson s (1941) observations in rabbits suggest that this may be an over simplification This author found that stretching of the cervix results, after a short delay, in increased myometrial activity similar to that seen after intravenous injection of oxytocin and that this phenomenon is abolished by spinal section (at T 7), or by electrolytic destruction of the pituitary stalk

That this increased uterine activity at the time of mating may be due to the release of oxytocin is supported by the observation, in several species, of milk ejection accompanying mating (Hammond 1936, Harris & Pickles, 1953 Campbell & Petersen 1953 VanDemark & Hays, 1953) Similarly manipulation of the vulva *the cervix or the uterus in the goat (Andersson 1951) and in the cow* (Tgetgel, 1926 Piana & Curto 1950, Usuelli Piana & Curto, 1952, VanDemark & Hays, 1951*a* 1953) also results in milk ejection

The concept of a neurohormonal reflex would be strengthened by the demonstra tion of an increase in the concentration of oxytocin in the blood at the time of mating No figures have as yet been published although VanDemark & Hays (1953) claim to have detected in the bovine an increase in milk ejecting activity of jugular blood at the time of mating We (Fitzpatrick & Hughes 1956) have recently made some preliminary investigations in cows under similar conditions

External jugular blood was collected from cows into heparinized polythene vessels over a two or a three minute period during which the uterus and cervix were stimul ated manually *per rectum* The blood was extracted by a modification of the methods of Vogt (1953) and of Bisset & Walker (1954) and finally assayed according to Holton (1948) using the superfused rat uterus Our results show that the oxytocic potency of the blood after our stimulation procedure ranged from 420 to 850 micro units/ml as compared with pre stimulation values of 120 to 300 micro units/ml This oxytocic activity was not blocked by atropine (1×10^{-7}) or lysergic acid diethylamide (2×10^{-8}) but was eliminated by treatment with 0 01 M sodium thioglycollate (Ames Moore & van Dyke 1950)

TABLE I

Oxytocin like responses

Species	Stimulus	Response	Author
Mare	Mating	Milk ejection	Hammond 1936
Man	Mating	Milk ejection	Pickles 1953
Man	Mating	Milk ejection	Harris & Pickles 1953
Man	Mating	Milk ejection	Campbell & Petersen 1953
Cow	Mating	Milk ejection	VanDemark & Hays 1953
Cow	Mating	Milk ejection	Campbell & Petersen 1953
Cow	Mating	Uterine motility	VanDemark & Hays 1952 1953
Bitch	Mating	Uterine motility	Evans 1933
Goat	Manual cervix	Milk ejection	Andersson 1951
Cow	Manual cervix	Milk ejection	VanDemark & Hays 1951*a*
Cow	Manual uterus	Milk ejection	Tgetgel 1926
Cow	Manual uterus	Milk ejection	Piana & Curto 1950
Cow	Manual uteru	Milk ejection	Usuelli *et al* 1952
Cow	Manual uterus/cervix	Milk ejection	VanDemark & Hays 1953
Cow	Manual vulva/cervix	Uterine motility	VanDemark & Hays 1951*b* 1954
Rabbit	Stretching cervix	Uterine motility	Ferguson 1941
Rabbit	Manual vulva	Uterine motility	Krehbiel & Carstens 1939
Rabbit	Increased intrauterine pressure	Uterine motility	Reynolds 1930
Cat	Increased intrauterine pressure	Uterine motility	Schubel & Gehlen 1933

Release of oxytocin at parturition

That oxytocin may be liberated as an essential part of normal parturition is suggested by the remarkable superficial similarity between spontaneous labour and that induced with oxytocin This view is also supported by the demonstration in the post parturient rabbit that electrical stimulation of the neurohypophysis results in increased uterine activity apparently due to release of a humoral agent and further that stretching the cervix of these animals evokes a neurohormonal reflex by which a stimulant substance is released from the posterior pituitary gland apparently in accordance with the requirements of labour (Haterius & Ferguson 1938 Ferguson 1941) The reports of Dicker & Tyler (1953) and Fromageot (this symposium) indicating that oxytocin is depleted from the neurohypophysis for some hours after labour in rats and dogs are in keeping with this hypothesis That oxytocin is liberated into the circulation during labour in women is indicated by Gunther s (1948) observation of the expression of milk coincident with uterine contractions

However attempts to demonstrate the presence of oxytocin in body fluids at the time of parturition have not given convincing results Increased oxytocic activity has been detected at this time in urine (Cockrill Miller & Kurzrok, 1934) and jugular blood (Bell & Morris 1934 Bell & Robson 1935) but the methods of extraction and

assay were not specific and it would seem necessary for these experiments to be re peated using the more specific methods available now

Experiments involving removal or lesions of the posterior pituitary and its hypo thalamic relationships, have in some hands shown that the neurohypophysial system plays some part in parturition Thus the work of Pencharz & Long (1933) in rodents Smith (1946) in monkeys, and Ranson and his colleagues in cats and guinea pigs (Fisher, Magoun & Ranson, 1938, Dey Fisher & Ranson, 1941) showed that hypo physectomy or electrolytic destruction of the pituitary stalk, resulted in prolonged labour and in maternal and foetal deaths These findings, moreover, are supported by observations of abnormally prolonged labour, in successive pregnancies in a woman with permanent diabetes insipidus (Marañon 1947)

These positive results, to my mind, carry greater weight than the corresponding negative reports (Allan & Wiles 1932 Selye, Collip & Thomson, 1933*a* and *b* Firor, 1933 Percharz & Lyons, 1934, McPhail, 1935, Dandy, 1940) which indicate in several species that stalk section or hypophysectomy fails to affect the course of subsequent parturition It has been suggested that these negative results may be explained as incomplete extirpations (Fisher *et al*, 1938) in this context the indica tions of regeneration made in the discussion of the preceding paper of this sym posium may be relevant On the other hand it has been reported that such lesions to the neurohypophysis may occasionally result in diabetes insipidus unaccompanied by detectable change in the course of parturition (Fisher *et al* 1938, Dey *et al* 1941 Dandy, 1940) Although these reports to some extent argue against the participation of the neurohypophysis in labour they are by no means conclusive, since effective delivery in the presence of diabetes insipidus, can be explained in terms of (*a*) com pensation by extra uterine components at parturition, or (*b*) by the survival of *sufficient neurohypophysial tissue to promote labour, although insufficient to* fully control water diuresis or (*c*) by invoking the concept of independent secretion of oxytocin and vasopressin The last possibility is particularly interesting in the light of the discussion to Dr Pickford s paper at this symposium Some such independence, in varying degrees has been seen after natural stimulation or electrical interference (Harris 1948 Peeters & Coussens 1950, Cross, 1951 1955, Kalliala, Karvonen & Leppanen, 1952, Abrahams & Pickford, 1954, and Olivecrona 1954)

Oxytocinase

It is perhaps appropriate here to recall that several workers have demonstrated that the incubation of added oxytocin and vasopressin with the blood of pregnant women leads to inactivation of the hormones (von Fekete, 1930, Werle Hevelke & Buthmann 1941, Woodbury Ahlquist Abreu Torpin & Watson, 1946, Page, 1946 Hawker, 1955) It is claimed that this inactivating power is also present at the time of parturition If these findings apply to endogenous oxytocin *in vivo* this would indicate that release of oxytocin from the neurohypophysis is not a factor in the process of birth unless one invokes the suggestion of Usuelli *et al* (1952) that the function *of circulating oxytocinase is merely to modify the* duration of action of endogenous oxytocin, thus preventing uterine spasm

Unfortunately the majority of workers have confined their attention to blood serum which in the light of present knowledge of non specific oxytocic activity of

serum, detracts from the significance of their results On this and on other points Tyler (1955) has recently criticized the quantitative aspects of previous work on oxytocinase and has reinvestigated the problem with improved techniques She has found that the 'oxytocinase activity of human plasma *decreases* over the last few weeks of pregnancy although vasopressin inactivating potency is unchanged Occasional persistence of plasma 'oxytocinase activity until the end of pregnancy she found to be associated with abnormally long labour A corresponding decrease in oxytocinase activity of tissues at the very end of pregnancy has also been reported by Sawyer (1954) in the rat

These more recent results change the significance of oxytocinase in relation to parturition (Nixon & Smyth 1956) Its function, if any, may be to facilitate the maintenance of pregnancy for the greater part of gestation this protection being withdrawn at the appropriate time

THE NATURE OF UTERINE RESPONSE TO OXYTOCIN

It is of interest to consider certain pharmacological aspects of the response of the uterus to oxytocin in relation to the requirements of parturition and seminal transport

Sensitivity

We have been impressed by the exquisite sensitivity of the superfused uterus of the rat in oestrus which responds to 25 micro units oxytocin This corresponds to a total dose of approximately 5×10^{-11} grams or 10^{10} molecules of du Vigneaud s polypeptide (Livermore & du Vigneaud 1949 Pierce Gordon & du Vigneaud 1952, du Vigneaud Ressler Swan Roberts Katsoyannis & Gordon 1953) In Table II is indicated in grammes and in molecules the corresponding sensitivity of this preparation to adrenaline nor adrenaline, acetylcholine and 5 hydroxytryptamine Only reports on high sensitivity to these substances have been included—in one case

TABLE II

Uterine sensitivity in vitro
(Oestrous rat uterus)

Drug	Minimum concentration g /ml	Molecular weight	Molecules per ml	Authors
Nor adrenaline	$7\ 5 \times 10^{-6}$	170	$3\ 7 \times 10^{15}$	Gaddum *et al* 1949
Acetylcholine	1×10^{-8}	163	$3\ 7 \times 10^{13}$	Fletcher 1953
Adrenaline	1×10^{-7}	184	$3\ 4 \times 10^{14}$	Gaddum *et al* 1949
Adrenaline	1×10^{-10}*	184	$3\ 4 \times 10^{11}$	Holzbauer & Vogt 1954
5 hydroxytryptamine	2×10^{-9}	174	$7\ 2 \times 10^{12}$	Garven 1956
Oxytocin				
30 μU /ml	$3\ 8 \times 10^{-11}$	1005	$2\ 3 \times 10^{10}$	Gaddum & Hameed 1954
100 μU /ml	$1\ 25 \times 10^{-10}$	1005	$7\ 7 \times 10^{10}$	Fitzpatrick 1956

* This sensitivity was exceptional

'exceptional' sensitivity (Holzbauer & Vogt, 1954)—but despite this, it is obvious from the Table that sensitivity to oxytocin is as great or greater than to any other substance, particularly when expressed in terms of molarity

In vivo sensitivity to oxytocin, of a similar or even greater order to that seen *in vitro*, can be deduced from available figures for plasma volume and for the minimum effective dose given intravenously in man, as shown in Table III

TABLE III

Uterine sensitivity to oxytocin in vivo

Species	State	Dose i v	Calculated plasma *Conc /ml*	Authors
Man	Pregnant 38 w	100 mU	30 μU	Woodbury *et al* 1946
	Pregnant 39 w	100 mU	30 μU	Nixon & Smyth 1956
	Term	50 mU	15 μU	Theobald *et al* 1948
	Term	20 mU	6 μU	Cantarow 1954
	Term	10 mU	3 μU	Nixon & Smyth 1956
	Term	12 mU	4 μU	Theobald *et al* 1948
	Term	5 mU	1 5 μU	Ratzan & Schulman 1955
Cow	Pregnant 38 w	1000 mU	40 μU	Fitzpatrick 1956
	Term	250 mU	10 μU	Fitzpatrick 1956
	Oestrus	500 mU	20 μU	Fitzpatrick 1956

This remarkable sensitivity particularly at times of special interest such as parturition and oestrus, carries the implication that to produce physiological responses only very small quantities of oxytocin need to be liberated into the circulation The effective concentration of oxytocin in the blood under these conditions may not be very much greater than that of vasopressin operative in the physiological control of water excretion

The response of the cervix uteri to oxytocin

It is commonly accepted that the sensitivity of the myometrium to oxytocin varies with changes in sex hormone secretion in an apparently purposeful manner Thus uterine reactivity to oxytocin is usually increased when under the influence of oestrogens and in many species depressed by progesterone (Knaus 1934, Robson, 1947, Reynolds, 1949 and others) These generalizations have been accepted as applying to uterine muscle as a whole Differences in response of different parts of the organ were not considered until the observations of Newton (1934 1937) which focused attention on the differences in reactivity of the cornua and cervix

There is now wide agreement in all species studied (Table IV) that the cervix uteri contains muscle fibres which can contract independently of those of the corpus or cornua This applies to spontaneous contractions (Karlson 1949, Adler *et al*, 1944,

TABLE IV

Species in which independence of cervical contractions from corpus (or cornual) contractions has been described

Species	Method	Author
Rabbit	*in vitro*	Bonnycastle & Ferguson 1941
Rabbit	*in vivo*	Adler Bell & Knox 1944
Rabbit	*in vivo*	Schofield 1949 1952
Guinea pig	*in vitro*	Newton 1937
Guinea pig	*in vivo*	Adler Bell & Knox 1944
Rat	*in vitro*	Newton 1937
Cat	*in vivo*	Adler Bell & Knox 1944
Goat	*in vitro*	Newton 1934
Cow	*in vivo*	Fitzpatrick 1951
Sheep	*in vivo*	Fitzpatrick 1951
Monkey	*in vivo*	Ivy Hartmann & Koff 1931
Man	*in vivo*	Karlson 1949
Man	*in vivo*	Schild 1951
Man	*in vivo*	Woodbury *et al* 1947
Man	*in vitro*	Danforth 1954

Fitzpatrick, 1951) to those following nerve stimulation (Schofield, 1952) and to responses to drugs and hormones including posterior pituitary hormones (Sauter 1948, Newton 1934 1937 Bonnycastle & Ferguson 1941 Adler *et al* 1944 Fitzpatrick 1951 Schild *et al* 1951)

Newton s early *in vitro* work showed that the isolated cornual muscle from the pregnant goat (1934) and the guinea pig (1937) contracted to low concentrations of oxytocin (400 micro units/ml) although under the same conditions cervical muscle from the same animal failed to respond to concentrations 100 times as great In contrast adrenaline stimulated the cervix but not the cornu This reciprocal pattern of response was also observed *in vitro* with cornual and cervical muscle of the parturient rabbit by Bonnycastle & Ferguson (1941) Such a pattern of response to oxytocin —absolute cornual dominance—is of course that appropriate to the process of parturition However this simple relationship between cervix and cornua was not found *in vivo* by Adler *et al* (1944) in the rabbit guinea pig and cat Oxytocin elicited contractions of both cornua and cervix and although quantitative differences were detected they were not pronounced

Our own observations are an extension of these and were made chiefly in ruminants and less extensively in pregnant women Ruminants have anatomical advantages over laboratory animals (and man) in the facility and the fidelity with which simultaneous intracervical and intrauterine recordings can be made This account is confined to results obtained in cows although similar results were obtained in sheep and in a small number of observations in man The recording method used was that described by Schild *et al* (1951) and employs small intracervical and intrauterine

balloons, coupled by water transmission, via a double co axial cannula, to linearly magnified mercury manometers writing frontally on kymographs

In the bovine, posterior pituitary hormones elicit contraction of both cervix and corpus but the reactivity of the two structures varies independently However, this lack of parallelism between responses of cervix and corpus does fall into an orderly pattern when considered in relation to the prevailing hormonal conditions of the animal

Responses in pregnant animals The magnitude of the response of the corpus to oxytocin increases slowly throughout most of the second half of pregnancy, and this accelerates dramatically just before parturition No such augmentation is seen with the cervix however the reactivity remaining constant or even diminishing

Since we recorded intrauterine and intracervical pressures simultaneously and independently it permits us to express the magnitude and direction of the intra uterine pressure gradient at the height of drug action, as the algebraic difference in the pressures in the two cavities This is illustrated in relation to duration of pregnancy in Fig 1 For simplicity, only responses at one dose level are shown, similar variation

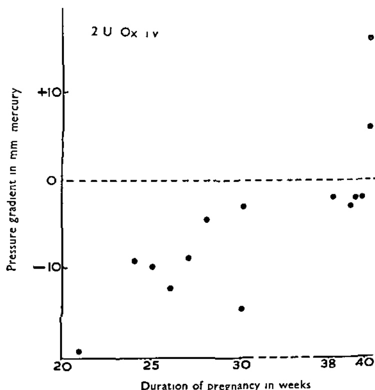


Figure 1

The gradient of pressure from corpus to cervix recorded after intravenous injection of a standard dose of 2 U oxytocin is shown in relation to duration of pregnancy This gradient is given by the algebraic difference between the pressures recorded simultaneously from the two cavities
Points above the dotted *zero line (positive values) indicate a greater pressure* in corpus than in cervix points below the line (negative values) indicate the reverse

was seen at all doses studied At mid pregnancy cervical response is dominant but this decreases in late pregnancy and, in animals studied less than thirty six hours before delivery the pressure gradient changes in favour of the corpus These changes are further indicated in Fig 2 in which simultaneous cervical and corpus responses are compared in the form of log dose—response curves for two experiments one at mid pregnancy and the other at term (40 weeks) Thus the response of the uterus as a whole is in favour of retention at mid pregnancy and of expulsion immediately before parturition

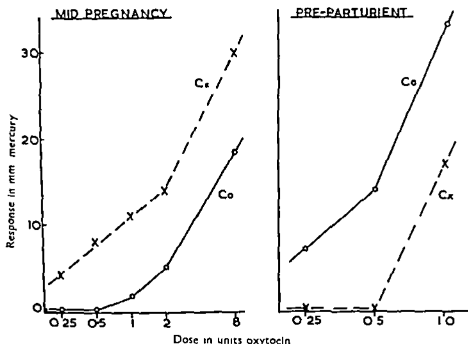


Figure 2

Simultaneously recorded responses of corpus (Co) and cervix (Cx) are plotted against dose of oxytocin (i v) at two stages of pregnancy
At mid pregnancy (22 weeks) the cervical responses are consistently greater than those of the corpus whilst in a cow 36 hours before delivery the corpus responses are the greater

These findings of a change in pattern of response immediately before parturition are in keeping with some earlier results which previously have appeared contradictory Robson (1933) and Russell (1943) each compared the *in vitro* reactivity to Pituitrin shown by strips of muscle obtained at Caesarian section from the fundus and the lower uterine segment in man Russell found the lower uterine segment strips more reactive than the fundal, whereas Robson found the fundal muscle dominant Russell s material however was obtained from women at term but not in labour, whereas Robson s were from women in active labour The dissimilar results of these two workers may possibly be explained on the basis of a change in the pattern of

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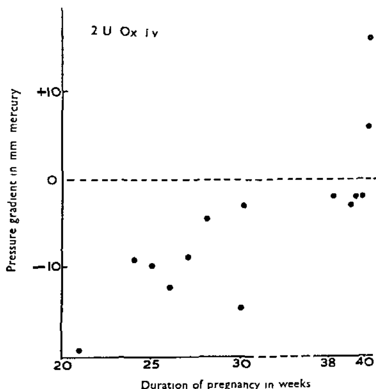


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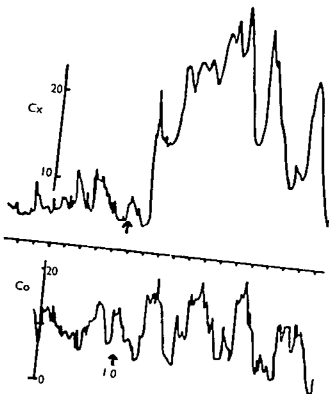


Figure 4

Simultaneous kymograph records of cervical (Cx) and corpus (Co) responses to 1 0 U oxytocin i v in a cow in oestrus showing cervical dominance

Time intervals 30 sec Pressure scale in mm of mercury

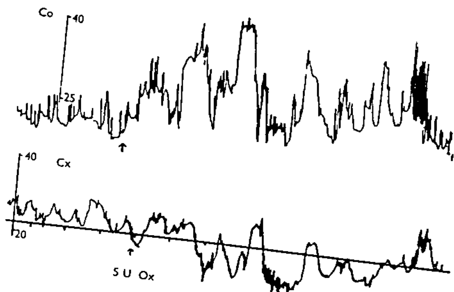


Figure 5

Simultaneou kymograph records of corpus (Co) and cervical (Cx) responses to 5 0 U oxytocin i v in a cow du ing the luteal phase of ovarian activity showing corpus dominance
T me ntervals 1 min Pressure scale in mm of m rcury

response immediately before the physiological onset of labour, similar to that seen in the bovine over the last forty eight hours of pregnancy

Response in non pregnant bovines In the non pregnant cow there is a marked variation in reactivity of the cervix to oxytocin it is low at mid cycle (luteal phase) and high during the oestrogen phase or after administration of oestrogens The corresponding variation in reactivity of the corpus is less pronounced, so that the intra uterine pressure gradient is largely a function of cervical response This gradient is shown in relationship to the ovarian cycle in Fig 3 Cervical dominance is marked at oestrus and corpus dominance at mid cycle, as illustrated in Figs 4 and 5

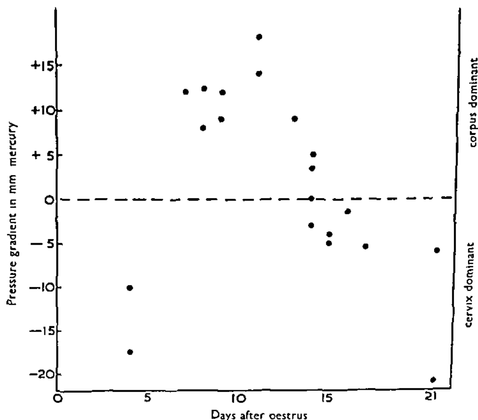


Figure 3

The pressure gradient from corpus to cervix after oxytocin is plotted against stage of ovarian activity in the non pregnant cow

The concept of differential reactivity of corpus and cervix to oxytocin gives two contrasting relationships in the intact animal At oestrus the responses combine to favour retention of uterine contents and propulsion of fluid from cervix to corpus i e a mechanism appropriate to sperm ascent In contrast to this immediately before parturition the combination of responses favours expulsion of uterine contents as appropriate to delivery of the young

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It is worth recalling Ferguson's (1941) interesting explanation of the co ordinating mechanism for successive births in pluriparous species This author has shown that in the parturient rabbit, mechanical stretching of the cervix results in uterine con tractions attributed to the reflex release of oxytocin, whilst dilation of the vagina provokes a sympathetic nervous response In the same species, it has also been shown (Bonnycastle & Ferguson, 1941) that *in vitro* the pattern of cervical and cornual response to oxytocin favours uterine emptying whereas that to adrenaline favours retention of uterine contents Thus presentation of a foetus against the cervix will liberate oxytocin to accelerate delivery, whilst its passage into the vagina will liberate adrenaline and temporarily retard the advance of the next member of the litter The implications of this are not confined to pluriparous species, in particular in man there is evidence that excessive sympathetic discharge during labour may interfere with the normal development of fundal dominance, resulting in cervical dystocia (Sauter 1948)

Our results, in another species *in vivo* are in keeping with the views expressed by Ferguson

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Discussion

Chairman Sir S Zuckerman

Cross I would like to make a point regarding the effect of adrenaline in antagonizing the action of oxytocin on the uterus and mammary gland which has been referred to in this and in the previous paper There is no evidence that adrenaline blocks the central release of oxytocin although it certainly can block the peripheral effect of the hormone on the mammary gland and on the uterus The central inhibition of

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shown that a negative pressure develops in the uterus of the mare at the time of mating, in keeping with the concept of fluid being forced up the uterine tract and not downwards

Some degree of inhibitory response of uterine muscle to sympathetic discharge and to adrenaline applies in my opinion, to most species Dr Cross suggested that some of VanDemark s recordings of uterine contraction could be attributed to sympathetic nervous reflexes rather than oxytocin The only known motor innervation of the bovine uterus is sympathetic and the response of the intact bovine uterus to adrenaline *at the time of oestrus*, I have found to be predominantly inhibitory and not contractile Cupps & Asdell (*J Animal Sci* 3, 351 1944) working with *in vitro* techniques also found that when under the influence of oestrogen the uterine muscle of the bovine was inhibited by adrenaline

Cross Some of my records of uterine response to hypothalamic stimulation look remarkably like those published by VanDemark

Fitzpatrick In which species is this?

Cross The rabbit

Dale The rabbit has a purely motor innervation through the sympathetic nerves

Cross In the rabbit the response is both motor and inhibitory

Dale But predominantly motor

Cross On stimulating the sympathetic centres of the hypothalamus one sees initial contraction followed by inhibition

Dale I was referring rather to direct stimulation of the sympathetic nerve as having a purely motor effect

Zuckerman That must be the final path! The hypothalamus is a long way from this

Dale Stimulating the hypothalamus does not ultimately differentiate between a nervou effect and a hormonal effect

Has Dr Fitzpatrick made any observations using vasopressin? Does the response of the bovine uterus to the subsidiary oxytocic activity of vasopressin vary in parallel with that of the activity of oxytocin?

Fitzpatrick There is an increase in reactivity of the bovine uterus to vasopressin over the last two weeks of pregnancy which is not paralleled by a corresponding increase in reactivity to oxytocin Conversely the sudden augmentation of response to oxytocin seen immediately prior to parturition is not seen with vasopressin It is possible to estimate the relative potency of oxytocin and vasopressin in these experiments and from mid pregnancy until the thirty eighth week (i e 2 weeks before term) oxytocin is between 7½ and 10 times as potent as vasopressin After the thirty-eighth week the ratio changes in favour of vasopressin i e the ratio tends towards unity Thirty four to thirty six hours before parturition there is an increase in reactivity to oxytocin which swamps that to vasopressin so that the ratio rises to 20 to 1 or more After parturition reactivity to oxytocin is lost more rapidly than that to vasopressin and

oxytocin release that occurs during emotional disturbance of the milk ejection reflex might be due to adrenaline but there is no proof, it could equally well be due to a purely nervous inhibition Dr Fitzpatrick s direct evidence of the secretion of oxytocin into the bloodstream following manual stimulation of the cervix is valuable since some of the previous evidence of American workers (VanDemark & Moeller, *Amer J Physiol* 165, 674, 1951) is not fully convincing In the cow the latent period found by these workers was less than 10 sec which seems to be too short to be explained in terms of a neurohormonal pathway, since even intravenously injected oxytocin has a latent period of about 15 sec It is possible that the uterine contractions recorded by the American workers could have been entirely nervous in origin Ferguson (*Surg Gynec Obstet*, 73, 359, 1941) for instance in the rabbit was able to produce uterine contractions by dilation of the vagina even after the spinal cord had been sectioned to eliminate the possibility of reflex oxytocin secretion I have recently confirmed this observation

Fitzpatrick I have found in the bovine too that adrenaline may have a dramatic antagonistic effect on oxytocin induced uterine contraction Further this is seen both *in vivo* and *in vitro*, which eliminates the possibility of the effect being an indirect one

Dr Cross made reference to the experiments of VanDemark and his co workers I have no information on the latent period between mating stimulus and myometrial response other than that obtained by examination of the published tracings, and these are not clear on this point

Cross I have heard directly from Dr VanDemark confirming that responses were obtained in bovines within 10 sec of cervical stimulation, both from the uterus and from the mammary gland The milk ejection reflex in the cow usually takes some 40 sec There is no known nervous motor path whereby milk ejection may be initiated but it is very difficult in this type of experiment to exclude the possibility of milk ejection artefacts, associated with changes in the posture of the cow

Dale Are there any figures for the rate of ascent of sperm in the guinea pig uterus?

Fitzpatrick Only of those of Florey & Walton (*J Physiol (Lond)* 74, 5P 1932) based on a fistula technique

Dale The point is that the innervation of the guinea pig uterus as far as I know is entirely inhibitory Otherwise it might seem more likely that a nervous mechanism would explain this rapid ascent of the sperm it would not be so easy to imagine that a hormone, in very small doses, would elicit such a co ordinated, antiperistaltic wave, as would seem to be necessary

Fitzpatrick I know of no direct evidence of such a wave of 'antiperistalsis but I do not see that it is necessarily impossible to consider an antiperistaltic wave if one accepts a response at other times in the form of a peristaltic wave

Dale It would perhaps be possible to suppose that if an antiperistaltic activity is normally present, then oxytocin might merely intensify it

Fitzpatrick There is some further evidence in agreement with my observations of a differential pressure gradient in the uterus Millar (*Aust Vet J* 28, 127 1952) has

Release of vasopressin and oxytocin in response to drugs

by

J M WALKER

Department of Pharmacology University of Oxford

IT is known that the neurohypophysis can be stimulated by a number of drugs Table 1 gives a list of some of the important ones This list may not be comprehensive

TABLE I

Substances which release vasopressin and oxytocin

	Vasopressin	Oxytocin
Ether (Ginsburg & Heller 1953)	+	
Phenobarbitone (de Bodo & Prescott 1945)	+	
Pentobarbitone (de Bodo & Prescott 1945)	+	
Acetylcholine (Abrahams & Pickford 1954)	+	+
Nicotine (Burn Truelove & Burn 1945)	+	
Morphine (de Bodo 1944)	+	
Yohimbine (Fugo 1944)	+	
Ferritin (Baez Mazur & Shorr 1952)	+	
ATP (Dexter Stoner & Green 1954)	+	

and it contains only the names of those for whose stimulant action there is reasonably good experimental support At first sight the drugs in the list appear so diverse that it is difficult to draw any conclusion at all They include some but by no means all of the anaesthetics nicotine like drugs an analgesic and various others This diversity might imply that their stimulant actions were exerted in different ways or even at different sites Indeed there is evidence that their modes of action are not all the same for the stimulant action of acetylcholine is dependent on the state of the cholinesterase in the supraoptic nucleus while that of morphine is not (Pickford 1953) However there is one fact which is suggestive namely that nicotine and acetylcholine (by a nicotinic action) are effective Now we know that the anti diuretic hormone and oxytocin are released as it were reflexly as a result of certain stimuli such as coitus and suckling We know also from the work of Pickford and her colleagues (Pickford 1947 Duke Pickford & Watt 1950) that small amounts of acetylcholine or of the anticholinesterase DFP when injected into the region of the supraoptic nucleus cause a transient antidiuresis It seems possible therefore that the nucleus is analogous to a peripheral autonomic ganglion in that it possesses a synapse at which acetylcholine is the transmitter and which is stimulated by nicotine The second point of interest about the list is that very little appears to be known about

the ratio approaches unity again for a short while, before returning eventually to the usual value of about ten to one I have shown recently (*J Pharm Pharmacol* 8, 403, 1956) that in the intact animal, parenteral magnesium chloride treatment increases reactivity of the bovine uterus to vasopressin but not to oxytocin, and it may be relevant that there is a rise in the magnesium concentration in serum in this species a short time before parturition (Moodie, Marr & Robertson, *J comp Path* 65, 20, 1955) It is possible, although at present pure speculation, that this physiological increase in magnesium concentration may be related to the early increase in vasopressin reactivity

Croxatto I want to comment on the oxytocinase activity of the blood during pregnancy in human beings We made observations some years ago which indicate that this activity increases progressively during the last month

Noble When Page (*Amer J Obst Gynec* 52, 1014 1946) first published his account of human serum in pregnancy inhibiting oxytocin we repeated and confirmed his experiments Dr A Eiger (unpublished observations) however, showed that although incubation with serum apparently destroys the activity of added Pitocin, the oxytocic activity returns if the mixture is left in the refrigerator overnight Thus the supposed destruction of oxytocin is better referred to as a reversible inactivation We think this probably indicates a pharmacological rather than a physiological process

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the release of oxytocin If we had information on this point, we should be able to calculate the ratio of oxytocin to vasopressin in the secretions of the gland in response to drugs, compare it with the ratio in the secretions produced by electrical and physiological stimulation, and thus perhaps gain some knowledge about the mode of release of the two hormones It is these two points, the behaviour of the synapse and the release of hormones in response to drugs, that I want now to consider in detail

METHODS

1 *Estimation of hormone content of the neurohypophysis*

It is possible to study the depletion of the neurohypophysis of its hormones, and this has been done with success in order to find out what happens when the supraoptico hypophysial tract is cut, when hypertonic saline is given or water withheld, and under various other conditions (for references see Heller, 1955) But as Dr Ginsburg has emphasized, the method has the disadvantage that it does not distinguish *per se* between the rate of the release of the hormones and their rate of formation for the amount of active substance in the gland at any moment must depend on these two variables For instance, Ginsburg & Brown (1956) found that when rats were anaesthetized with pentobarbitone and a large amount of blood was withdrawn from the circulation, there was actually a significant increase of pressor and oxytocic activity in the neurohypophysis although presumably in the meantime antidiuretic hormone had been released by the gland This suggests that the formation of the hormones had been accelerated under these conditions

2 *Effects of drugs in the whole animal*

With this method, the drug is administered to the animal, and the effect of the consequent output of neurohypophysial hormone is observed It is the method that has been most frequently used but is liable to give the most misleading results A serious difficulty is that the compound which is being investigated may have effects of its own on the target organ (e g the kidney) which is being used to study the activity of the neurohypophysis, or it may mask in some way the action on that organ of the hormones that are being secreted At Oxford, Dr G W Bisset and I have worked chiefly with rats, and we have found that changes in the animals blood pressure produced by some of the drugs we were testing had distorting effects on urine flow which complicated the antidiuretic action we were studying Two examples of the importance of the blood pressure in this regard may be mentioned First if nicotine is injected into a group of rats at the beginning of a water diuresis, the chart of excretion of urine shows two peaks The first peak, which occurs soon after the injection, is we believe caused by a rise of blood pressure, because a similar peak can be produced by a purely pressor agent like tyramine and because it is abolished if the nicotine is preceded by hexamethonium which prevents its pressor action The second example is illustrated in Fig 1 which shows the arterial blood pressure and urinary flow, simultaneously recorded of a rat anaesthetized with ethanol It can be seen how exactly the pressor or depressor actions of the substances injected were matched by changes in the rate of formation of urine

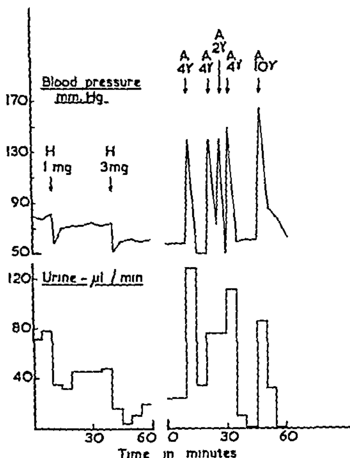


Figure 1

Rat anaesthetized with ethanol. Arterial blood pressure and urine flow. Arrows mark intravenous injections of hexamethonium (*H*) and adrenaline (*A*)

3 *Estimation of vasopressin and oxytocin in body fluids*

For these reasons it is important if possible to extend results obtained by method 2 by assaying directly the hormone content of the body fluids. Urine is not satisfactory since it has not been found possible to get rid of non specific substances which contract the isolated uterus and therefore interfere with the assay of oxytocin. It seemed best therefore to do the assays on blood itself. The antidiuretic hormone is rapidly destroyed in its passage round the body and we have therefore done our assays on blood from the external jugular vein which in the rat is the main path of venous drainage from the hypophysis. There is no lack of suitably sensitive methods for assaying antidiuretic hormone and the preparation we have used is that of Ames & van Dyke (1952). But in order to assay oxytocin we had to evolve a method by which blood was treated with acid ethanol and then concentrated by evaporation under reduced pressure and which allowed us to test the resulting extract on the

isolated uterus of the rat (Bisset & Walker, 1954) There are two points about the results obtained with this method In the first place, in order to obtain sufficient material on which to carry out the assays, it was necessary to take quite large amounts of blood Ginsburg & Heller (1953) have shown that withdrawal of blood may itself be a potent stimulus for production of antidiuretic hormone, and this fact complicates the interpretation of our findings The second point is that there are present in the extracts oxytocic factors that are not of pituitary origin, for it has not always been possible to destroy all the oxytocic activity by sodium thioglycollate One of these factors, which we think was potassium, is easily got rid of by dialysis Another factor, however is sometimes present Since Armstrong, Keele, Jepson & Stewart (1954) showed that if blood was allowed to come into contact with a water wettable surface such as glass, a pain producing substance was formed which was also oxytocic we tried the experiment of collecting blood in syringes and beakers made of polythene We have found that under these conditions non specific factors seldom appear This method has been applied by Dr Bisset to experiments on man about which I hope he will tell us at the end of the paper

RESULTS

We were led to do the experiments to which I shall refer by some earlier work on the excretion of chlorides When commercial whole posterior lobe extract is injected subcutaneously into rats, there is not only an antidiuresis but also an increase in the total chloride excretion When oxytocin is injected, there is a chloruresis without an antidiuresis and the major part of the chloruretic effect of whole lobe extract has therefore been ascribed by many workers to the oxytocic hormone I am aware that there is some doubt about this, and that the whole picture is now altered by the very interesting results which Miss Pickford has described to us at this meeting Moreover it would obviously not be possible to interpret the increased chloride excretion after the administration of drugs without investigating the renal function of the treated animals Nevertheless we thought it would be of interest to see whether it was possible to correlate the size of the antidiuresis produced by a drug with the level of antidiuretic hormone in the jugular blood on the one hand and the size of the chloruresis with the level of oxytocin on the other Accordingly nicotine was injected into groups of hydrated rats water and chloride excretion was measured, and the effects were compared with those of commercial extract It was found (Fig 2) that nicotine in a dose which was equal in antidiuretic power to a given dose of posterior lobe extract caused a rather greater chloruresis It was possible therefore that after nicotine there would be both vasopressin and oxytocin present in the jugular blood and on *investigation this proved to be so There was more oxytocin than vasopressin*, and the mean ratio was 1 8 though the figures varied considerably from one animal to another (mean level of oxytocin 5 7 mU per ml vasopressin 3 1 mU per ml, see Fig 3) The levels of both oxytocin and vasopressin were significantly raised above those of the control animals

If, as I suggested earlier the supraoptic nucleus can be regarded as analogous to a peripheral autonomic ganglion a blocking agent such as hexamethonium might be expected to block the stimulant action of nicotine However we have found (Bisset & Walker, 1953) that even in large doses which completely block both pressor and

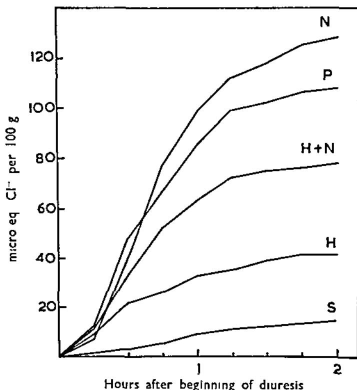


Figure 2

The effect of drugs on the chloride excretion in rats N = nicotine H = hexamethonium P = pituitary (posterior lobe) extract In the test groups drugs were given in doses of equal antidiuretic potency Control groups (S) received an injection of saline (Bisset & Walker 1956)

convulsive effects of nicotine hexamethonium does not block its antidiuretic action Some other blocking agents do not do so either (Supek & Eisen 1953) There is a slight but significant diminution of the chloruretic effect of nicotine when it is preceded by hexamethonium (see Fig 2) but this diminution cannot be confidently ascribed to a central inhibitory action for although the mean concentration of oxytocin in the blood after hexamethonium and nicotine is lower than that after nicotine alone the difference is not significant at the 5 per cent level (P = approximately 0·1)

In the course of this work it was found that hexamethonium itself given in the large dose of 5 mg per 100 g had an antidiuretic effect equal to that of nicotine but that this was accompanied by only a very small chloruresis (Fig 2) This antidiuretic effect can be shown not to be due to a delay in the absorption of water from the gut It could be of course and very likely is at least partly due to a haemodynamic effect on the kidney But when the jugular blood was assayed it was found

that the antidiuretic hormone content was significantly raised above that of the controls—raised in fact to about the value found in rats that had received nicotine—whereas the oxytocin was not altered It is possible that hexamethonium stimulated the neurohypophysis indirectly by causing a profound fall of blood pressure Figure 3 summarizes these results

When we were taking the blood from the jugular veins, it was convenient to have the rats anaesthetized, and in the experiments hitherto described the anaesthetic used was ethanol which was given by mouth in a large volume of water, so that the rats were hydrated to a degree similar to that of the rats in the earlier experiments on chloride excretion Ethanol was chosen for two reasons First, though it interferes

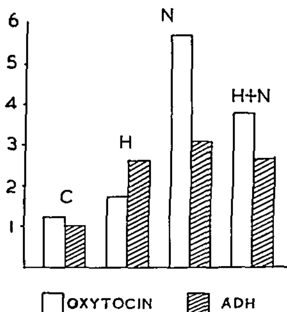


Figure 3

Concentrations of antidiuretic hormone and oxytocin in the jugular venous blood of rats under ethanol Values in mU per ml blood *N* = nicotine *H* = hexamethonium *C* = controls (Bisset & Walker 1956)

with the output of antidiuretic hormone under physiological conditions previous work had shown us that it did not abolish the antidiuretic effect of nicotine Second it was thought that ethanol would not itself cause release of antidiuretic hormone In fact it can be seen (Fig 3) that the level of hormones in the blood of these controls was about 1 0 mU per ml This accords with Dr Ginsburg s finding that the fourth and fifth millilitre of blood withdrawn from an anaesthetized rat have quite high levels of antidiuretic hormone since we withdrew from 4 to 6 ml, our mean value of 1 0 mU per ml does not seem unreasonable In some more recent work, ether was used instead It was found that when nicotine was injected into rats under ether, the oxytocin level of the jugular blood was not significantly different from that in the blood of animals under ethanol but the mean level of antidiuretic hormone was

1 0 mU per ml in the former against 3 1 mU per ml in the latter Thus in these experiments ether, which itself is known to cause release of antidiuretic hormone, apparently diminished the power of nicotine to do so without interfering with the release of oxytocin

CONCLUSIONS

From these results certain conclusions are possible In the first place if hexamethonium reaches the supraoptic nucleus in what would be an effective concentration and yet does not block the action of nicotine the synapse there must have pharmacological properties quite different from those of peripheral autonomic ganglia Second nicotine produces a secretion of both oxytocin and antidiuretic hormone in the rat but in a ratio of the former to the latter much lower than is thought to be usual in other animals Thus estimates of the ratio vary from about 5 in the rabbit (Harris 1948) to about 20 in the dog (Abrahams & Pickford, 1954) whereas we have found a ratio of 1 0–1 8 There are a number of possible reasons for this discrepancy The conditions of our experiments were different in that our rats were anaesthetized whereas the animals used by most other workers were not Again we used nicotine whereas others used other forms of stimulation But let us suppose for a moment that the low ratio in the rat is characteristic of the species Now it is not known what function oxytocin has in the male, or in the non pregnant or non lactating female animal Dr Pickford has discussed the possibility that it helps to control the excretion of sodium by the kidney In most species the amount of oxytocin which has to be given in order to affect sodium excretion seems to be very much larger than the amount of vasopressin which is antidiuretic and it has therefore sometimes been assumed that this action of oxytocin is an incidental one of no physiological significance But as Dr Pickford has pointed out it is not correct to calculate doses on the arbitrary basis of the amounts of activity in commercial extracts They should rather be calculated on a basis of the activities apparent when the animal s own neurohypophysis is in action If this is a correct way of looking at the problem it might be expected that an animal which secreted comparatively small amounts of oxytocin would possess a kidney which was sensitive to the electrolyte controlling action of the hormone It would thus be most interesting to know how the rat s kidney reacts to small amounts of purified oxytocin and vasopressin

But knowledge of the actions of drugs on the neurohypophysis might have an even wider significance Let us suppose that the hypothalamic nuclei are analogous not to a peripheral ganglion as I suggested at the beginning but to a nucleus which is also part of the autonomic system namely that of the third cranial nerve This nucleus has sometimes been thought of as being under continuous inhibitory influences from higher levels in the central nervous system When the influences are removed by sleep or anaesthesia the nucleus becomes active and the pupil constricts A similar set of conditions might apply to the supraoptic nucleus Thus in a conscious animal which was conserving water impulses from osmoreceptors would keep the nucleus in a state of activity and antidiuretic hormone would be continuously released During a water diuresis however the nucleus would be inhibited by influences from elsewhere in the central nervous system and in the absence of osmoreceptor activity would remain inactive The analogous situation with respect to the

third nerve nucleus would be in the conscious animal in the light, with pupil con stricted, on the one hand, and the conscious animal in the dark, with pupil dilated, on the other Under anaesthesia inhibitory influences would be removed and the supraoptic nucleus would become active, causing antidiuretic hormone to be pro duced In the present state of our knowledge perhaps this can hardly amount to more than a fanciful analogy But it might point the way to explaining some of the earlier work by Bonsmann (1930) for instance, who showed in dogs that some hypnotics such as phenobarbitone prevent the normal response to water administration, while others, such as paraldehyde, do not The effect of an anaesthetic on water metabolism would depend on whether or not it removed these inhibitory influences from the supraoptic nucleus Moreover, a hypnotic could easily act at two sites at once, it could remove extrahypothalamic inhibition and thus promote secretion of neuro hypophysial hormones and at the same time it might by acting directly on the nucleus, interfere with the stimulant action of a drug like nicotine These two modes of action would explain why phenobarbitone which itself releases antidiuretic hor mone, nevertheless diminishes the antidiuretic action of nicotine (Supek & Eisen 1953), and also why ether as we have shown though itself a releaser of antidiuretic hormone reduces the amount of the hormone released by nicotine Conceivably morphine might be in the special position of stimulating the nucleus direct, the available evidence suggests that its action in constricting the pupil is due to a stimul ant effect at or near the third nerve nucleus (for discussion see McCrea, Eadie & Morgan 1942) rather than to a removal of cortical inhibition and Pickford (1953) has shown that morphine produces antidiuresis when injected direct into the supra optic nucleus

All this must remain mere speculation until further work is done, but it may be useful in suggesting the lines on which such work might profitably be undertaken First, we need reliable information about the action of anaesthetics, based if possible on a direct determination of the level of hormones in the blood Second, we should know what is the reaction of the hypothalamo hypophysial system to newer sub stances such as reserpine and chlorpromazine, which are thought to act primarily on the hypothalamus Third we must find out more about the release by drugs of oxytocin, a subject about which we are still very ignorant

Finally, there is one aspect of the problem which has not so far as I know, yet received attention In the rat the neurohypophysial hormones are partly associated like histamine and 5 hydroxytryptamine with particles of mitochondrial size (Pardoe & Weatherall 1955) and recently Dr L Martini and I have found that the same is true for the dog It has been shown that histamine is released from its particles by histamine releasers (Mongar & Schild, 1954) and that 5 hydroxytryptamine is released in the same way from its particles by reserpine (Giarman 1956) It will be very interesting to see if vasopressin or oxytocin can be liberated from their particles by pharmacological agents and if so whether these agents stimulate the neuro hypophysis in the living animal

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Discussion

Chairman Sir S Zuckerman

Bisset I should like to report on some clinical extensions of this work which I carried out with Dr J Lee Blood was withdrawn into a siliconed syringe by puncture of the internal jugular vein in patients at operation within 10 to 15 min of the induction of anaesthesia with sodium thiopentone Concentrated alcohol extracts were

assayed for antidiuretic activity by intravenous injection in the Ames and van Dyke preparation and for oxytocic activity on the isolated rat uterus The sensitivity of the assay rats for antidiuretic hormone ranged from 10 to 30 μU /ml blood In only 2 of 9 cases was antidiuretic activity detected the concentrations were 12 and 20 μU /ml blood Satisfactory oxytocic assays were carried out in 8 cases, the concentrations of oxytocin varied from 1,200 to 2 300 μU /ml blood with a mean of 1 600 μU /ml The activity was abolished by sodium thioglycolate

We hope to determine whether the level of oxytocin in the blood is raised during parturition and lactation and conversely whether certain types of uterine inertia and of dystocia and painful engorgement of the breast during lactation, are associated with a deficient secretion of the oxytocic hormone

Determination of both oxytocin and vasopressin in a single specimen of a body fluid may have further interesting applications For example, assay of oxytocic and antidiuretic activity in the blood after damage to, or stimulation of, the supraoptic and paraventricular nuclei might indicate the relative importance of these two structures in the secretion of oxytocin and vasopressin

We wish also to determine the ratio in which the hormones are released after stimuli such as injection of nicotine, since Dr Pickford has shown that it is necessary to know this ratio before determining the effects of the hormones on such functions as renal sodium excretion

Heller I was very interested in Dr Bisset s remarks I can—to some extent—confirm his findings on the antidiuretic activity in human internal jugular blood We (Heller & Schmieden unpublished observations) drew blood by direct puncture of the vein in local anaesthesia but we have results in three subjects only The antidiuretic activity in two subjects was less than 20 μU /ml and in a third case less than 11 μU / ml I am mentioning this not only because it agrees with Dr Bisset s observations but also because it stresses the fact that only very small amounts of antidiuretic activity can be expected in the blood of normal adults Results like these make the statements of various authors that several milliunits per millilitre may be found in the peripheral blood of normal human beings rather difficult to understand

van Dyke What volume of blood did you take from the external jugular vein in rats?

Walker We took between four and six millilitres We were conscious of the fact that the withdrawal of such a volume would influence the hormone level but there appeared to be no correlation between the volumes of blood taken and the concentrations we found There might have been a correlation if we had had information about the blood pressure of the rats

van Dyke The significance of these relatively higher concentrations of oxytocin is difficult to appraise I don t think that even the use of the thioglycollate test necessarily proves that the substance is oxytocin All it shows is that the active substance has such a chemical constitution that its biological activity can be destroyed by reduction

I wanted also to ask what significance you attach to the change in the ratio (*V/O*) of the hormones in the blood You spoke of its being raised in some instances and not in others

Walker The answer to the first point is that without definite chemical identification we cannot be absolutely certain that the substance is oxytocin We think it is because it behaves in every way that we have tried like the pituitary hormone We have never found any in peripheral blood (though we have not done many experiments on this) and it appeared in jugular blood in amounts which did not seem to us to be unduly high considering the amounts of antidiuretic hormone Ginsburg & Heller found Finally we looked for it in the jugular blood of hypophysectomized animals and although it was still present (possibly because our hypophysectomies were technically at fault) the amount was very greatly reduced If these results were entirely due to chance, it seems to me unlikely that we should have such differences between one series and another

Ginsburg How do you know that you have not got any nicotine in your blood extract?

Walker Because we dialysed it In any case, nicotine does not elicit uterine contraction

Gross I want to ask Dr Walker whether he has measured the sodium output under the influence of his various pituitary stimulating agents and if so whether it parallels chloride output?

Walker We did not do that because we had no flamephotometer at hand at that time I think that Dr Pickford showed yesterday that chlorides move in the same way as sodium

Zuckerman Do you imagine that the same nerves which are involved in neurosecretion also transmit nervous impulses?

Walker Yes I have always thought so

Dale Do I understand that you have extracted whole blood? Is there any reason for that?

Walker We had the impression from the literature that if we had centrifuged the red cells off we might have lost some of the activity We found that extracting whole blood was quite satisfactory and so we stuck to it

Dale If you are extracting whole blood you are loading your extract with all sorts of substances both from the red cells and from other formed elements I wonder if there is any evidence that the hormone is stuck to the red corpuscle

Fitzpatrick Using an extraction procedure similar to that of Bisset and Walker I found that added Pitocin is not absorbed onto red blood cells On the other hand during the time required for centrifugal separation of the plasma there is a decrease in activity—suggesting that there is some direct inactivation

How important is 5 hydroxytryptamine in these extractions? In taking whole blood one is also taking the platelets which Hardisty & Stacey (*J Physiol* (*Lond*) **130**, 711 1955) found to contain most of the 5 hydroxytryptamine We find that added 5 hydroxytryptamine comes through the extraction procedure in sufficient quantity to interfere with oxytocin assays on the rat uterus It may be dangerous to use lysergic acid diethylamide in the final assay procedure to eliminate the effect of

5 hydroxytryptamine since lysergic acid diethylamide also inhibits uterine response to oxytocin

Walker In cases where we thought that we might have had an effect from 5 hydroxytryptamine in our extracts, we found that doses of lysergic acid diethylamide which were sufficient to abolish the effect of 5 hydroxytryptamine did not affect the responses to oxytocin

Pickford If you were to allow 5 hydroxytryptamine to continue in its contact with the uterus, you would soon differentiate between it and the oxytocin because the preliminary contraction is not maintained as it is with oxytocin

Zaidi We have used a modification of Dr Walker s extraction procedure and I would like to ask him whether he ever found unspecific antidiuretic substances in his extracts Professor Croxatto has mentioned that mere acidification will produce antidiuretic activity in blood

Walker No We usually did the oxytocic assays first and in cases where we did suspect a non specific oxytocic action there was no evidence of a non specific antidiuretic effect

Zaidi To refer to your analogy between the neurohypophysial system and the third cranial nerve we noticed that when rats go to sleep, the urine flow often falls, and I wonder whether this cannot also be explained by this analogy

Walker I do not want to go back to this analogy too much, but if you were to press me and ask for an answer, then it would be yes

The adrenal-neurohypophysial interrelationship

by

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INTRODUCTION

This subject will lead into complexities of homeostatic mechanisms from which clear concepts have not yet emerged We will begin therefore by outlining four groups of widely accepted facts which serve as the basis for the problem (1) The antidiuretic hormone (vasopressin) is the main mammalian humoral agent acting to inhibit water excretion and prevent fluid loss through the kidney under certain conditions it enhances sodium excretion (2) The 17 hydroxy (hydrocortisone like) hormones of the adrenal cortex depending upon circumstances, may act either to enhance or inhibit water and sodium excretion (3) The aldosterone like adrenal hormones are the main humoral agents acting to inhibit sodium excretion and because of that fact must to some degree tend to cause water retention they too however *can* act as water diuretics (4) The secretion of antidiuretic hormone is regulated in part by the osmotic pressure of the extracellular fluids and probably to some degree by extracellular fluid volume changes The secretion of aldosterone is regulated likewise partly by electrolyte concentrations in body fluids and again perhaps by extracellular fluid volume Unlike aldosterone the secretion of the hydrocortisone like steroids is under the control of corticotrophin

These facts show that the adrenal cortex and neurohypophysis affect related phenomena and are regulated in part by similar influences The problem is to define the nature of the interrelationship between them The attempt to do so began in 1938 and has not yet succeeded fully

In 1938 Silvette & Britton called attention to some of the essentially antagonistic actions of the neurohypophysis and adrenal cortex and emphasized their probable functional significance In elaborating this concept (e g Corey & Britton 1941) they dealt with gross phenomena and did not attempt to specify the mechanisms through which these overall antagonistic influences might operate * Since that time numerous authors have dealt more or less comprehensively with the subject in attempts to correlate admittedly difficult and conflicting data into comprehensible working hypotheses (e g Lloyd 1952 Heller 1952 Pitts 1952 Montastruc 1954 Morel 1955 Chester Jones 1956) The attempts of the senior author and his associates to force knowledge from all sources as it existed in 1949 and 1950 into some

* It is now apparent that in some respects the corticoids may enhance rather than antagon e the action of neurohypophysial hormones (See Sect II)

degree of coherence were summarized in two publications (Gaunt, Birnie & Eversole 1949, Gaunt, 1951) Their suggested cause and effect relations were admittedly tentative and tenuously bound In some respects they have proved too fragile to withstand subsequent critical scrutiny Let us look, then, briefly at some past work in the field and deal especially with more recent observations The need for brevity will require evaluations more dogmatic than are justified either by the evidence at hand or the authors self confidence, unfortunately only a fraction of the relevant literature can be cited Since work in the lower vertebrates is being covered by Dr Chester Jones in a subsequent paper of this symposium, we omit all references to this field Also, since it is not relevant to our main theme, no further note will be made of the important new work of Ingle & Li (1955) implicating vasopressin as an essential agent in the repair of the deficient muscle work performance of adrenalectomized animals

I PROBLEMS CONCERNING THE NATURE OF THE ADRENAL NEUROHYPOPHYSIAL RELATIONSHIP

The facts at hand permit the postulation of an almost infinite number of sites and means of interaction between the hormones of the two glands in question Many of these possibilities have been explored to some degree with a resultant large literature that is notable for its lack of agreement Some of the questions at issue follow

A *Circulating levels of antidiuretic hormone in adrenal insufficiency*

Several investigators have presented evidence indicating that increased quantities of some circulating antidiuretic substance (ADS) exist in adrenal insufficiency The relation of this ADS to the antidiuretic hormone, however, is not clear, largely because of question concerning specificity of the assay methods used (Ames & van Dyke, 1952 van Dyke Adamsons & Engel 1955) and contradictory findings concerning facts involved Nevertheless questions concerning the identity of such substances should not be allowed to obscure the fact that the phenomena which have been observed are not illusory Any satisfactory solution of the problem must account for the presence in adrenal insufficiency of an excess or easy release of some substance(s) which in certain tests resembles the neurohypophysial antidiuretic hormone (Birnie, Eversole Boss Osborn & Gaunt, 1950, Slessor 1951 Lloyd 1952 Leaf & Mamby, 1952 Mirsky Stein & Paulish, 1954*a* 1954*b*, Ginsburg 1954, Montastruc 1954)

B *Relation of filtration rate to renal actions of corticoids and antidiuretic hormone*

It is generally agreed that the antidiuretic hormone influences water and electrolyte excretion without affecting the glomerular filtration rate (G F R) It is also agreed that the sodium retaining action of corticoids can be effected independently of the G F R There has, however, been much confusion concerning the superimposed relation of the definite G F R increasing action of the corticoids to such phenomena (Boss Birnie & Gaunt 1950, Rosenbaum Davis & Ferguson 1951, Pitts 1952 Davis & Howell, 1953, Stribling & Spurr, 1954 Skillern Corcoran & Scherbel 1956) The question is important because if some of the effects of corticoids e g effects on water diuresis, are due indirectly to changes in the G F R there would be no need to invoke altered function of the neurohypophysis in their explanation

Otherwise a neurohypophysial participation might logically be suspected The nature of the problem is such that definitive evidence is difficult or impossible to obtain in view of the possibility that changes in G F R too small to be measurable by available methods can affect electrolyte and water excretion (Selkurt, 1954) Nevertheless impressive evidence suggests that there are effects on water excretion attributable to corticoids or their lack which are not explicable by changes in renal hemodynamics (Rosenbaum *et al*, 1951, Sala Sereni & Ballabio 1951 Stribling & Spur 1954 Skillern, *et al*, 1956, Dingman & Thorn, 1955 Dustan Corcoran & Farrell, 1955 Ikkos, Luft & Olivecrona, 1955 Garrod Davies & Cahill, 1955) This does not by itself implicate participation by the neurohypophysis as several authors concerned have pointed out but does open a door to such possibilities An additional complicating factor in such studies is that experimental work on hypophysectomized animals and in human subjects with diabetes insipidus have a well known limitation of lack of certainty that neurohypophysial function is completely abolished (cf Sect IV *B* 3)

The concept that the decreased tubular reabsorption of water (increased free-water clearance) sometimes clearly associated with the administration of corticoids represents a direct tubular action has not fared well in recent studies (e g Skillern *et al* 1956) Alternatively it has been proposed that the potassium deficiency induced by corticoids leads to an inability of the renal tubule to reabsorb water (Corcoran & Dustan 1956)

C Effect of corticoids on the sensitivity to antidiuretic hormone and on thirst

Some time ago it was suggested (Birnie *et al* 1950, Lockett 1952) that adrenalectomized animals are hypersensitive to exogenous vasopressin The subsequent demonstration that adrenalectomy delays the rate of inactivation of vasopressin (Sect III) seemed to provide a sound basis for such observations Nevertheless others have failed to find that such a hypersensitivity exists in man (Chalmers & Lewis 1951 Dingman & Thorn 1955) Whatever may be true in adrenal deficiency states it is clear from numerous observations beginning with those of Ragan Ferrebee Pfyfe Atchley & Loeb 1940 (Winter 1952 Swingle Fedor Ben Maxwell Baker & Barlow 1953 Dustan *et al* 1955 Conn 1955) that excess corticoids including aldosterone reduce the responsiveness to vasopressin (cf Figs 1 and 2) although the opposite has been reported in hypopituitarism (Stribling & Spurr 1954) The mechanisms involved are not well established but one factor seems to be indirect the corticoids cause a polydipsia which seems to be an important element in the altered fluid exchanges in chronic experimental situations (above references Gaunt 1951 Skillern *et al* 1956 Ikkos *et al* 1955)

D Relation of antidiuretic hormone and corticoids to electrolyte excretion

In pharmacological experiments various types of interactions between various corticoids and vasopressin on sodium and potassium excretion are demonstrable some examples are illustrated in Sect II It can be questioned whether such effects of vasopressin are direct or indirect (Leaf Bartter Santos & Wronz 1953) Despite considerable effort however no well documented story has yet emerged showing convincingly a physiological counterpart of these pharmacological observations

(Murphy, 1950, Black & Thompson, 1951, Chalmers, Lewis & Pawon, 1951, Holland & Stead, 1951, Lauson, 1951, Maxwell & Breed, 1951, Pitts, 1952, Davis, Howell & Hyatt, 1954, Cole, 1955) Although some recent work such as that of Anslow & Wesson (1955) and Chester Jones (1956) gives promise of clarification, there is still scant evidence of *deficiency* phenomena which would be anticipated if the neurohypophysial hormones had important effects on electrolyte metabolism

II CORTICOID VASOPRESSIN INTERACTION PHARMACOLOGICAL CONSIDERATIONS

There have been many studies in which vasopressin and some adrenal cortical preparation were given simultaneously (e g , Ragan, *et al* , 1940, Corey & Britton, 1941, Sartorius & Roberts 1949, Winter, 1952, Swingle, *et al* , 1953, Morel 1955) We have recently extended work of this sort to try to distinguish between the different actions of several corticoids at varying dosages on the response to Pitressin* At the present time it is obviously not permissible to speak of adrenal influences

Effect of hydrocortisone (F) on the response to Pitressin

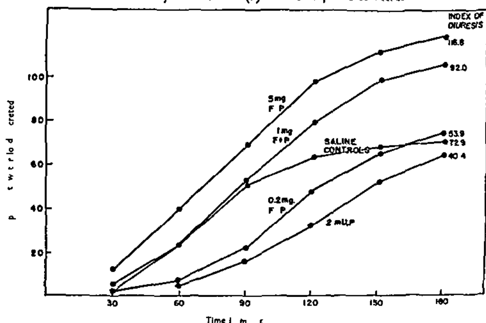


Figure 1

Showing the effect of graded doses of hydrocortisone on the antidiuretic effect of Pitressin The Index of Diuresis represents in cm the area under each curve

* We utilized simple acute experimental procedures in normal male rats Fasted animals were given a hydrating dose 5 ml /100 g of 0 2 per cent sodium chloride solution by stomach tube together with a standard subcutaneous injection of 2 mU Pitressin/100 g The effect on the response to Pitressin of varying doses of the several steroids given subcutaneously two hours before Pitressin was then determined in terms of water sodium and potassium excretion over a period of 180 min In order that values for water excretion might be expressed as one figure the area under cumulative curves of water excretion was plotted (see Krieger & Kilvington 1940) and designated the index of diuresis One centimetre on the ordinate represented excretion of 10 per cent of the fluid load and one centimetre on the abscissa represented 10 min time

without specifying the particular type of adrenal hormone involved The results illustrated in Figs 1 and 2 are based upon the means of all animals receiving any particular treatment Untreated and Pitressin treated controls were run simul taneously with all treated groups and hence served as a check on possible erroneous conclusions due to day to day variations

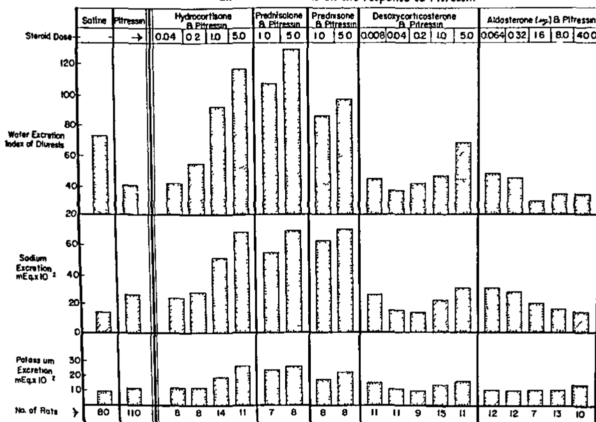


Figure 2

Show ng the effects of var ou co t co ds on the response to a standard dose (mU) of P tress n in expe ments of 180 min durat on The Index of D ures s ep e e t ng the amount of water excreted s illustrated in F g 1 and defined in the footnote on p 236

The results are complex and indicate that no simple rule governs the observed responses Hydrocortisone in doses of 200 μg or higher antagonized partially or completely the antidiuretic effect of Pitressin* and at the higher doses (1–5 mg) it actually caused dehydration (Fig 1) On the other hand hydrocortisone augmented Pitressin induced natriuresis and to a lesser degree kaliuresis Prednisone and predni solone had quantitatively different effects For instance prednisolone was the most active compound in stimulating water excretion whereas prednisone enhanced

* We a e at a loss to correlate these find ngs with those of Winter (1952) who fourd co t one to enhance the antid uret c effect of P t es n whereas desoxyco t coste one acetate antagonized t

sodium excretion relatively more than water (These actions of the Δ' compounds are of interest because of their relative absence of effect on sodium and water metabolism under most conditions of clinical use)

Desoxycorticosterone in the presence of Pitressin had no effect on water excretion except to enhance it at high dosage—an action definitely weaker than that of the glucocorticoids In contrast to the glucocorticoids, desoxycorticosterone in small doses reduced sodium excretion whereas large doses were without effect This apparent anomaly might result from a rise of the G F R produced by large doses of desoxycorticosterone

Aldosterone was available only in microgram doses In these amounts it had no effect on water excretion except for a suggestion of enhancement, which could not be established statistically of the antidiuretic action of Pitressin at certain doses Other work (Renzi Renzi Chart & Gaunt, 1956) leads to the presumption that aldosterone in larger dosage would have a hydrocortisone like diuretic action greater than that of desoxycorticosterone Aldosterone in amounts of 1 6 μg or more inhibited the natriuretic action of Pitressin qualitatively like the intermediate doses of desoxycorticosterone When used in minimal effective dose ranges aldosterone seemed to have about 25 times the antinatriuretic activity of desoxycorticosterone This corresponds closely to the relative sodium retaining activity of these two steroids in some other but not all kinds of test situations (Gaunt, Renzi & Chart 1955)

It is clear from this study that one cannot simply speak of corticoid vasopressin antagonism The effects of Pitressin may be either antagonized, enhanced or unaltered by a corticoid depending upon the steroid used its dose, or the variable measured Such pharmacological observations do not tell us with certainty what to expect when these hormones are secreted in physiological amounts under variable conditions They do suggest however that no simple general rules apply and that segregation of influences involved under clinical conditions will be a discouragingly difficult problem

III RELATION OF ADRENAL TO INACTIVATION OF ANTIDIURETIC HORMONE

The work of Birnie (1953) and Ginsburg (1954) seems to establish unequivocally on the basis of both *in vivo* and *in vitro* experiments that adrenal hormones support the mechanisms possibly enzymatic and/or nutritional (Lloyd 1952) which are responsible for the rapid inactivation of vasopressin After adrenalectomy the half life of circulating exogenous vasopressin was doubled a process partially blocked by cortisone but not by sodium chloride therapy

Such observations together with suggestive evidence of high blood levels and accelerated release of antidiuretic hormone make it probable that adrenal insufficiency results in a *hyper vasopressinism* One cannot be certain however because of lack of definite evidence in such cases that the neurohypophysis does not reduce its rate of secretion and thus compensate homeostatically for a reduced rate of hormone destruction

IV INTERACTION BETWEEN RELEASE MECHANISMS OF ADRENAL AND NEUROHYPOPHYSIAL HORMONES

Insofar as the adrenal and neurohypophysial hormones act antagonistically it might be anticipated that a hypersecretion of one would incite a hypersecretion of the other Within the range of physiological dosage, pharmacological evidence to support such an idea has not been forthcoming On the other hand reciprocal function might be attained by one hormone suppressing the secretion of the other It is in this general field that the greatest progress has been made recently

A *Vasopressin like substances and release of corticotrophin (ACTH)*

In the search for the neurohumour which presumably traverses the hypothalamico hypophysial portal circulation to cause ACTH release much attention has been directed to the possibility that vasopressin is the substance concerned Exogenous vasopressin in large dosage (like large doses of many other things) can cause ACTH release (Nagareda & Gaunt 1951 Kimura 1954 McCann & Brobeck 1954 Sobel, Levy Marmorston Schapiro & Rosenfeld *et al* 1955 Martini & Morpurgo 1955 Shibusawa, Saito Fukuda Kawai & Yoshimura 1955, Sayers & Burks 1955) although under certain conditions the opposite may occur (Itoh & Arimura 1954) That it does so in what might be considered physiological amounts* has not been shown, this and other more critical evidence has led to doubt that vasopressin could be the neurohumour involved (Nagareda & Gaunt, 1951 Harris Lloyd & Lobotsky 1953 Munson & Briggs 1955 Guillemin & Rosenberg 1955) Much interest awaits attempts to extend the reports suggesting that the hypothalamic neurohumour in question is a substance present in posterior pituitary extracts but different from vasopressin or oxytocin (Guillemin & Hearn 1955 Saffran Schally & Benley 1955 Porter & Rumsfeld 1956 Swingle Brannick Barrett, LeBrie & Parlow 1956 Guillemin, Hearn Cheek & Housholder 1956)

It is also possible that the antidiuretic hormone affects corticoid excretion by indirect routes, e g expansion of the extracellular fluid volume (Davis *et al* 1954) or simple excess hydration (Nagareda & Gaunt, 1951) Beck Dyrenfurth Giroud & Venning (1955) have shown an increased excretion of aldosterone only during the period *after withdrawal* of Pitressin therapy a result suggesting the operation of indirect influences

B *Effect of corticoids on neurohypophysial activity*

Cavallero Dova & Rossi (1954) working in Heller s laboratory were the first to report that the hormones of the adrenal cortex affected the pituitary content of antidiuretic hormone This content was reduced by adrenalectomy and restored to varying degrees by salt desoxycorticosterone acetate and cortisone—the latter apparently being the most effective These findings are apparently in some disagreement with those of Diamond (1952) They are not entirely consistent in detail but are consistent in principle with our observations noted below

*Physiological amounts is an even more elusive term in this case than usual Even if physiological levels of vasopressin could be well estimated in the systemic circulation that would not necessarily imply that the same concentration occurs in the hypothalamico-hypophysial portal vessels if vasopressin arising from the hypothalamus was actually acting as a neurohumour at such a site

The authors approach to the problem has been of two types (*a*) the vasopressin like content of extracts of the posterior pituitary and hypothalamus was assayed in various experimental situations (Lloyd and associates in the Syracuse laboratories), (*b*) an attempt was made to quantitate the amount of stainable neurosecretory material in the neurohypophysis under various conditions (Gaunt, Chart & Antonchak (1956) in the CIBA laboratories)

1 *The effect of adrenal and pituitary hormones on the content of antidiuretic substance in the pituitary and hypothalamus* The content of antidiuretic substance of posterior pituitary and hypothalamus was estimated by determining the decrease in urine flow caused by the intravenous injection of homogenates of these tissues into rats anaesthetized with ethanol All of the donor animals were sacrificed by decapitation under Nembutal anaesthesia It had previously been shown that the content of hypothalamic and posterior pituitary antidiuretic substance was essentially unchanged by Nembutal Both lobes of the hypophysis were routinely included in the material assayed because numerous experiments under different conditions failed to demonstrate antidiuretic activity in the anterior lobe The results (Lloyd & Pierog, 1955, Lloyd & Oldford 1955 and unpublished data) are shown in Table 1 classified in terms of the following groups

Group I Antidiuretic substance (ADS) is present in large amounts in the posterior lobes (200–400 mU equivalents of vasopressin) and in lesser amounts in the hypothalami (10–20 mU equivalents) of normal rats Moderate dehydration produced a variable increase of hypothalamic but loss of pituitary antidiuretic activity Hydration resulted in slightly lower levels of hypothalamic activity Both procedures decreased pituitary ADS levels Preliminary observations in two cases suggested that intracarotid injection of hypertonic saline solution caused a marked depletion of all antidiuretic hormone stores

Group II Immediately after hypophysectomy the hypothalamus and blood contained very little antidiuretic activity but after a month or two the hypothalamic and blood levels increased significantly The secretory activity of the hypothalamus retained a certain flexibility as demonstrated by the decrease of ADS following a moderate degree of dehydration and the accumulation of ADS upon hydration These findings suggest that in the presence of an intact hypothalamico neurohypophysial system efficient mechanisms of synthesis and release easily accommodate to moderate changes in antidiuretic hormone requirement, whereas in the hypophysectomized animal supply more readily reflects changes in demand

The presence of the adenohypophysis in the absence of the neurohypophysis influences the hypothalamic ADS content Low levels of blood ADS and very little hypothalamic ADS were found in rats with diabetes insipidus resulting from neuro hypophysectomy after a postoperative interval sufficiently long to permit reorganization of the hypothalamus and release of ADS in totally hypophysectomized animals (Lloyd & Pierog, 1955) The explanation of the role of the anterior lobe can only be speculative at the moment The lowering of hypothalamic ADS is not simply the result of the rapid water exchange which takes place in these animals because the levels of ADS are considerably lower than those in intact animals with a comparable rate of water turnover resulting from the use of a liquid diet

TABLE I

The antidiuretic hormone like content of the hypothalamus and posterior pituitary of rats
(From Lloyd *et al* 1955 and unpublished)

	Treatment	ADS content mU Pitressin equiv ±S E Hypothalamus	Post pit	No of rats
Group I Altered hydration in intact animals	Untreated controls	12 4±–3 9	302±74	14
	No water—48 hr	25 0±10 7	100±72	8
	Intracarotid hypertonic saline*	6 6	100	2
	Liquid diet 48 hr	9 7± 0 7	—	6
Group II Hypophysectomy (Hpx) and neurohypophysectomy	Hpx 3 to 8 days	2 0	—	6
	Hpx 30 days	10 0	—	6
	Hpx 30 days—no water for 48 hr	5 0	—	6
	Hpx 30 days—liquid diet for 48 hr	24 0	—	6
	Neurohpx 30 days—diabetes insipidus	2 0	—	6
Group III Stress and hydrocortisone in intact animals	Formalin stress†	2 5± 0 1	61± 24	6
	Hydrocortisone‡	50 0±24 6	414±195	6
	Stress+hydrocortisone‡	2 6± 2 26	2113	6
Group IV Stress and hydrocortisone in hypophysectomized animals	Hpx—30 days+stress	2 0	—	6
	Hpx—30 days+hydrocortisone‡	71 0±42 0	—	7
	Hpx—30 days+hydrocortisone‡ and stress	2 6± 0 73	—	6
Group V Adrenalectomy (Adx)	Adx—Salt free diet 2 days	4 9± 1 8	172± 60	6
	Adx—Salt free diet 2 days+hydrocortisone§	6 3± 1 6	307± 69	6
	Adx—Salt free diet 5 days	4 9± 2 0	91± 32	6
	Adx—Salt free diet 5 days+hydrocortisone§	8 3± 1 5	193± 70	7

* 2 ml of 2 5 per cent NaCl
† 0 2 ml of 10 per cent formalin subcutaneously 60–90 min before test
‡ 2 5 mg twice per day for total of 5 doses
§ 5 0 mg twice per day for total of 5 or 10 doses

Group III In intact animals the administration of a stress in the form of subcutaneous formalin results in decreased hypothalamic and posterior lobe ADS The administration of hydrocortisone caused a marked increase in ADS of both hypothalamus and posterior lobe of intact animals

Priming with hydrocortisone before formalin was given caused intact animals to accumulate large amounts of ADS in the posterior lobe while the hypothalamus became depleted

Group IV In contrast, hydrocortisone caused an increase of hypothalamic ADS in hypophysectomized animals but did not protect against depletion as a result of stress

These experiments could be interpreted as suggesting that a noxious substance such as formalin causes a movement of ADS out of the hypothalamus into the posterior lobe and thence into the blood stream in the intact rat and perhaps directly into the blood stream of the hypophysectomized animals Adrenal steroids do not prevent the migration from the hypothalamus but do seem to block release from the posterior lobe

Group V Adrenalectomized rats maintained on 1 per cent saline solution seemed to have relatively little change in ADS content of hypothalami or posterior lobes but due to technical difficulties reliable quantitative figures are not yet available It was only after depletion of salt and development of insufficiency that significant lowering of hypothalamic and posterior lobe ADS occurred If animals were maintained on a low salt diet, administration of very large amounts of hydrocortisone which would have caused accumulation of huge amounts of ADS in intact rats caused only partial return toward normal values The corollary experiments comparing the effects of desoxycorticosterone and NaCl with those of the 17 OH steroids are still in an early stage Our preliminary impression is that maintenance of animals in good health by either saline or DCA tends to prevent depletion of hypothalamic and posterior lobe ADS

In all of these experiments one consistent finding was that hydrocortisone caused increases in the amounts of ADS in the posterior pituitary relative to comparable controls whatever may have been its effect on hypothalamic stores of this substance Such observations are in general agreement with those cited below

2 *Effects of adrenal hormones on neurosecretory material** In our hands no measurable effect on this material could be obtained with mild or short term stimuli such as nicotine injections or short periods of dehydration We could not prevent depletion by giving 150 or 750 mU of Pitressin tannate although 2 5 U gave suggestive results Obviously the procedure as we used it is a less sensitive one than that of bioassaying total content of the tissue concerned Severe dehydration † however, caused marked

These experiments were done in collaboration with our associate Mr Ben Antonchak who was responsible for the histological work We are greatly indebted to Professor E Scharrer Albert Einstein College of Medicine for detailed advice and suggestions concerning techniques and procedures and to Professor J H Leathem Rutgers University for technical help

† Preliminary experimentation indicated that a simple dehydration procedure which would produce a distinct depletion of neurosecretory material in both intact and adrenalectomized animals was as follows mature non fasted male rats were given 3 ml of 5 per cent NaCl solution per 100 g body weight by stomach tube at 9 a m and this dose was repeated at 12 noon on the day the experiments started The animals were sacrificed at 24 hr i e 9 a m of the second day Depletion was negligible when animals were killed at 7 hr

depletion of the neurosecretory material as others have found (cf Scharrer & Scharrer, 1954) (Fig 3)

Effect of corticoids on the neurosecretory material of the neurohypophysis in rats

TREATMENT	Relative Density Of Neurohypophysis % Controls (50 60 70 80 90 100)	BALANCE/100: urine water	BALANCE/100: Na MEq x10⁻²	NO RATS
Controls Intact				46
Controls – ADX				9
Intact Dehydrated		-7.78	-0.65	50
Intact-Dehydrated F		-8.58	0.95	18
Intact-Dehydrated DC		10.2	0.90	25
ADX Dehydrated		5.64	0.50	25
ADX Dehydrated F		7.70	0.48	17
ADX Dehydrated DC		-6.70	0.20	17

Figure 3

General explanations in text The term Balance/100 represents the excretion of water and sodium greater or less than that administered during the 24 h dehydration period Units = ml or mEq /100 g body weight

There was slight if any, depletion of neurosecretory material in animals which developed spontaneous severe adrenal insufficiency from 7 to 21 days after operation This confirms the work of Sawyer & Roth (1953) and Rennels Russell & Drager (1955) but it does not agree with the findings of Eichner (1953) Neither did animals adrenalectomized for 18 hr show any differences from normal controls Adrenalectomized rats subjected to the 24 hr dehydrating procedure however showed a consistently greater depletion than intact animals (Fig 3) This was associated with an expected smaller urinary loss of water and sodium than in normal animals

Hydrocortisone in total doses of 7 5 mg (acting over a 40 hr period) prevented depletion clearly *in adrenalectomized rats and probably in intact ones* ($P = < 0\ 05$) Desoxycorticosterone (free alcohol) was without consistent effect in intact rats after adrenalectomy it prevented depletion but less effectively than hydrocortisone In

No food or water was allowed during the experiment Animals were kept in metabolism cages and the amounts of urine excreted measured at intervals the amounts of sodium and potassium excreted were determined for the 24 hr period

When steroid hormones were given the total dose was divided into three equal parts and injected as follows 4 p m on the day before the experiment (which was the time of adrenalectomy for operated animals) and with each of the two doses of hypertonic saline The total dose acted over a 40-hr period

Posterior pituitaries were fixed in Zenker Formol solution cut at 5μ and stained with Gomori Halmi Dawson aldehyde fuchsin The amounts of neurosecretory material were estimated by averaging the relative density of stainable material in 7 sections (450× magnification) from each gland The readings made on a Photovolt Densitometer and expressed in percentage terms are the figures shown in Fig 3

similar experiments 2.5 mg. doses of desoxycorticosterone were without effect and the same amount of hydrocortisone showed only suggestive protection. It is difficult to reconcile these results with the report of Billenstien & Leveque (1955) that desoxycorticosterone facilitates discharge of neurosecretory material from regenerated neurohypophysial tissue of hypophysectomized rats. For some curious reason, however, some intact animals in our series showed unexpectedly great depletion when given desoxycorticosterone. This was not a consistent finding but was possibly related to Billenstien & Leveque's results.

These experiments, together with those cited in the previous section, show that adrenalectomy and steroid hormones affect both the neurohypophysial content of antidiuretic material and the visible substances associated with it. This presumably means either that the corticoids inhibit release of this material, facilitate its synthesis or both. The choice of interpretation in such a case is one that has plagued students of the hormone content of glands for many years. Some work pertinent to the question is cited below.

3. *Effect of adrenal hormones on release of antidiuretic hormone.* Ginsburg (1954) provided evidence that adrenalectomy in animals facilitates the release of antidiuretic hormone. In retrospect that interpretation probably best explains previous data of

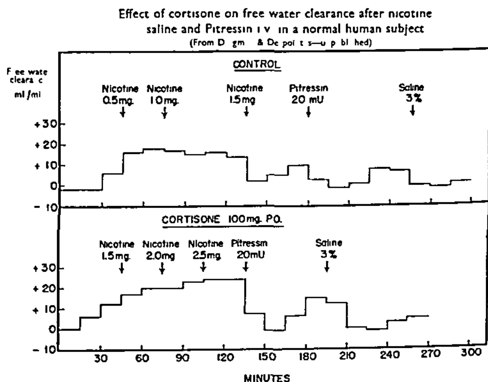


Figure 4

Showing an increased free water clearance and decreased responsiveness to nicotine under the influence of cortisone (given 105 min. before the test) in a subject maintained under a constant water load of 20 ml./kg.

Birnie, *et al* (1950) in which relatively high levels of circulating antidiuretic activity were associated with blood withdrawal in adrenalectomized rats Winter (1952) first stated that cortisone (in contrast to desoxycorticosterone acetate) might inhibit the release of antidiuretic hormone Recently the work of Dingman (1954) Dingman & Thorn (1955) and Dingman & Despointes (1956) indicates that cortisone, hydro cortisone and their Δ analogues have this effect in man

In studying patients with diabetes insipidus they concluded that in certain cases hypothalamic centres could release antidiuretic hormone in response to small doses of nicotine when this did not occur in response to hypertonic saline The antidiuretic response to nicotine was effectively blocked by cortisone given two hours prior to the nicotine In normal subjects the nicotine induced decrease in free water clearance was blocked by cortisone hydrocortisone and prednisolone whereas that to hyper tonic saline and Pitressin was not (Fig 4) The effects were independent of osmolar or electrolyte excretion The polyuria induced in diabetes insipidus patients with cortisone was associated with an increased free water clearance These results together with those of Sect IV *B* 1 are most easily interpreted on the basis that the 17 hydroxycorticoids inhibit some part of the mechanism for the release of the anti diuretic hormone

V CONCLUSIONS

A review has been made of work concerned with various possible sites and mechan isms of interaction between the hormones of the neurohypophysis and adrenal cortex Most of this work has failed to lead to clean cut conclusions of a widely acceptable sort To what extent for instance the abnormalities of water metabolism in adrenal *insufficiency or adrenal hyperfunction are due to some aberrant function* of the neurohypophysis is still not clear Recent work, however has established several apparently definite facts which although yet to be fitted into a comprehensive physio logical picture nevertheless represent distinct contributions toward that end This work which had its origin in numerous laboratories, may be summarized as follows

1 Different types of adrenal hormones have different effects on the responses to vasopressin Vasopressin actions may be antagonized augmented or unaffected by simultaneous steroid administration depending upon the nature of the steroid its dose and the variable measured Therefore generalizations in this regard about adrenal influences as such are not appropriate

2 The hydrocortisone like adrenal steroids under experimental conditions at least either inhibit the release or facilitate the synthesis of antidiuretic hormone and its visible neurosecretory substrate The former interpretation seems to be the pre ferred one although a combination of the two is not excluded A distinct difference exists possibly qualitative in nature between the effects of hydrocortisone and desoxycorticosterone in this respect

3 The maintenance of the mechanism in various tissues which accounts for the rapid inactivation of antidiuretic hormone is functionally dependent on hydro cortisone like adrenal steroids

4 The postulated neurohumoral substance of hypothalamic origin which causes

release of corticotrophin (ACTH) is probably closely related chemically to the known neurohypophysial hormones

Such findings suggest that the functional interrelationship between the adrenal cortex and neurohypophysis is a many faceted one The corticoids apparently affect neurohypophysial activity *per se* but in addition affect the response to neurohypophysial hormones and such related matters as their rate of inactivation A reciprocal aspect is that ACTH release, and hence certain aspects of adrenal function is probably under the control of hypothalamic neurohumours closely related to the known neurohypophysial hormones If all of this is the case, the relationship involved can not be reduced to terms of simple definition and generalization analogous to those between the trophic hormones and their target glands

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Discussion

Chairman H B van Dyke

Chalmers I would like to ask a question concerning Dr Lloyd s findings and that is whether the antidiuretic material was depleted in the adrenalectomized animals treated with DCA?

Lloyd We adrenalectomized animals leaving them for a week on 1 per cent salt and then did measurements of the antidiuretic hormone content of the hypothalamus and posterior lobe and could not show any real decrease We then decided that in order to get more definite changes we had to expose the animals to more severe conditions We put the animals on a salt free diet and found that we did get a reasonable depletion of the hormone content and with hydrocortisone we got a repletion We now have got about seven animals in each group that is to say animals which have been on desoxycorticosterone trimethylacetate plus salt and animals maintained on salt alone These animals were maintained for as long as three weeks there was moderate depletion but not nearly as great a depletion as in the animals which are allowed to become insufficient We have now a few experiments with animals given DCA and in contrast to cortisone we found very little increase in the antidiuretic activity in the hypothalamus or posterior lobe

van Dyke Do you feel that you have secured a significant lowering of the antidiuretic titres in the hypothalamus?

Lloyd For fifteen observations the standard error is less than ±20 per cent

Bargmann Did you get depletion after total adrenalectomy? Eichner (*Z Zellforsch* **37**, 406, 1953) obtained remarkable depletion of the neural lobe eighteen hours after adrenalectomy and I remember that Malandra and Corbetta (*Z Zellforsch* **39**, 318 1953) also observed some depletion

Gaunt We are aware that some but not all others had found depletion after simple adrenalectomy We did not The difference might depend upon the handling or strain of animals Certainly the adrenalectomized animal is susceptible to depletion after a stimulus such as dehydration

Heller I wonder if these observations do not fall into place when one considers the results of Ginsburg (*J Endocr* **11**, 165, 1954) who showed that haemorrhage is a more potent stimulus for hormone release from the neurohypophysis in adrenalectomized rats than in normal animals Dehydration seems also to be a more potent stimulus after adrenalectomy

Rossi Dr Gaunt stated that a stress by injection of formalin induced a marked depletion of antidiuretic hormone from the neurohypophysis I should like to point out that in experiments which we have done in Prof Heller s department (Cavallero Dova & Rossi *J Endocr* **10**, 228 1954) we observed a similar depletion of the hormone through the stress of sham adrenalectomy but this depletion disappeared within three to five days after the operation Concerning Dr Lloyd s report about the incomplete recovery of the antidiuretic hormone content in the neurohypophysis of adrenalectomized rats treated with compound F and sodium chloride, I would like to recall that we failed to find in the neurohypophysis of adrenalectomized rats after 5–7 days treatment with cortisone and salt any significant decrease in antidiuretic hormone as compared with normal control

Garrod I would like to ask Dr Gaunt a question regarding the experiment in which hydrocortisone was limiting the antidiuresis from Pitressin Has he got any evidence to suggest whether this is a direct antagonism at the renal tubular level or merely secondary to a rise in G F R ?

Gaunt In our experiments direct renal effects of the steroids could theoretically have explained the results We did not do the measurements to find out However, in some experiment not considered here today we got similar effect of steroids under conditions in which we could not detect (with our techniques) changes in G F R

Reiss *Dr Gaunt has raised the question as to whether the antagonism* he has found between hydrocortisone and the antidiuretic hormone has any physiological significance I would rather suggest that it has We can show it in psychiatric cases, particularly in depressive patients who excrete very little urine We have seen some who excrete only 220 ml urine per day If the depression improves the rate of urine excretion goes up by about one thousand per cent In such cases we have measured the rate of hydrocortisone excretion and found that when they excrete very little urine, there is only very little hydrocortisone in the urine and when they recover there is an enormous increase in 17 ketosteroid output

Heller Returning to the question of the hormone content of the neural lobe in adrenalectomized rats Dr Gaunt mentioned that some workers found an increase and others a decrease If I remember rightly Sawyer (Sawyer & Roth, *Fed Proc* **12**, 125 1953) for example measured the oxytocic content That was at a time when we were not aware that the hormones may be secreted separately and I think that his results may have to be regarded in that light

Sawyer That is absolutely right Another point is that our laboratory diet appears to have been relatively high in sodium content and when these adrenalectomized rats were maintained on this diet and tap water they survived for about two weeks They do lose a lot of weight and the plasma sodium levels are extremely low The hormone content of the neural lobe granted they were oxytocic assays, increased

In the earlier part you were talking about the enhancement of natriuresis with compound F Is this an effect of compound F itself or an enhancement of the action of Pitressin?

Gaunt Compound F will have the effect when given alone but it is greater when given with Pitressin

de Wardener I wonder if the adrenal is concerned with the finding that in dehydration the concentration of the urine is greater than after giving Pitressin This discrepancy becomes more marked with increasing hydration until in the case of obsessional polydipsia the patient may no longer be able to concentrate above the osmolarity of plasma however much Pitressin is given

Gaunt I do not know anything about such chronic states—if one hydrates with distilled water acutely an adrenal ascorbic acid depletion occurs Similar volumes of normal saline will not have any effect on this measure of adrenal activity

Ginsburg I am not happy about antidiuretic assays on gland extracts which may contain variable amounts of oxytocin particularly if these assays were done on rats It has been shown that the oxytocic principle can have a diuretic effect in rats and if the oxytocin content is changing in some unknown way it may effect the antidiuretic assay The other point I wish to make concerns Dr Gaunt s use of the word inactivation when referring to my work on the fate of Pitressin in adrenalectomized animals I would rather say clearance All that I could show was that after injection of a large dose of vasopressin the activity disappeared from the blood rather more slowly than in normal animals I do not know anything about what happened to the vasopressin when it was taken up by the inactivating tissues As to the physiological significance of this observation, I was very careful about its interpretation because it seemed to be possible that it may be due to a vascular effect if for example the renal vessels were very much more sensitive to the vasoconstrictor action of Pitres in in the adrenalectomized animal that could account for the failure of the kidneys to take up the material

van Dyke Does oxytocin have a diuretic effect after intravenous injection?

Ginsburg I do not know It has after subcutaneous injection but I do not think it has been tried after intravenous injection in rats

Comparative aspects of adrenocortical-neurohypophysial relationships

by

I CHESTER JONES
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Evolution of the vertebrates has been accompanied by elaboration of mechanisms which tend to preserve homeostasis One essential attribute of the successful vertebrate, or of the Craniata at least is the capacity to regulate water and ionic exchange The Myxinoidea apart which maintain in their plasma concentrations of inorganic ions equivalent to those of the surrounding sea water (Robertson, 1954) members of the different vertebrate classes enjoy a constancy of internal medium thereby withstanding the vicissitudes of environments varying from total immersion in fresh water to the dehydration of the desert From consideration of experimental work on a few species of mammals principally of the Eutheria, two glands of internal secretion namely the adrenal cortex and the neurohypophysis (and associated hypothalamic centres) have been assigned a major role in that equipage of the vertebrate body which maintains internal steadiness in the face of external change How far we are justified in extending the argument from mammals to include all vertebrates is not sure and there is little work in the field to help us (Chester Jones 1956*a* *b*) Some of this work I shall discuss later I should like in the first place however to consider some of the facts in the rat an animal on which much of our knowledge is based This part may be regarded as an appendix to Dr Gaunt s excellent paper in which he and his colleagues have clearly shown the present position of the problem of adrenocortical neurohypophysial relationships in mammals and have adduced helpful new data

MAMMALIAN STUDIES

I have used rats from which the posterior lobe of the pituitary was removed Neurohypophysectomy was done by the parapharyngeal approach and the posterior lobe removed without manifest disturbance of the anterior lobe of the pituitary All such operated animals developed a marked polyuria the water intake varying between 5 to 9 times that of the normal animal This temporary phase of diabetes insipidus lasted for up to three weeks when there was a decline The water intake of many animals fell to that of normal rats In the minority the decline was not so great steadying at a water intake of 4 to 5 times the intake of normal animals and maintaining this level for up to three post operative months beyond which I have not kept them Such animals are regarded as having permanent diabetes insipidus Examination of sections of the pituitary and hypothalamus of the rat with permanent

diabetes insipidus reveals an anterior lobe of normal appearance and degeneration of the hypothalamic supraoptic and paraventricular nuclei.

Apart from the polyuria (the specific gravity of the urine being 1·007 ± 0·003 compared with the normal figure of 1·051 ± 0·004) the rats with diabetes insipidus show no obvious physiological abnormalities. Thus, the growth rate is unaltered: for example one group of 34 male rats weighed 151·3 ± 2·7 g. at operation and 213·4 ± 4·4 g., 48 days later, while the control group of 22 rats weighed 192 ± 4·4 g. and 257·1 ± 5·2 g. over the same period. In female rats with diabetes insipidus the oestrous cycle is of normal duration. For example, one group of 7 females with permanent diabetes insipidus investigated for a month had a mean body weight of 202·95 ± 2·4 g., a food intake per 24 hr. of 16·13 ± 0·18 g., a water intake per 24 hr. period of 91·92 ± 1·09 ml. and a mean duration of oestrous cycle of 5·2 days. Six female controls over the same period gave body weight 229·50 ± 2·24 g., food intake 14·82 ± 0·31 g., water intake 21·06 ± 0·75 ml., and oestrous cycle of 5 days duration. It seems reasonable to suppose that any physiological difference found to exist between normal rats and rats with diabetes insipidus would depend primarily on the increase in fluid exchange of the latter.

TABLE I

The adrenal weights of male and female rats with diabetes insipidus compared with those of control animals
(Means ± standard error.)

	n	body weight g.	adrenal weight mg./100 g. body weight
(a) Neurohypophysectomized for one to two weeks			
Males with diabetes insipidus	19	210·52 ± 5·14	25·23 ± 1·02
Control males	39	224·59 ± 2·19	15·61 ± 0·78
Females with diabetes insipidus	10	184·50 ± 3·97	48·81 ± 2·14
Control females	19	193·42 ± 5·20	32·07 ± 1·20
(b) Neurohypophysectomized for two to three months			
Males with diabetes insipidus	28	233·75 ± 6·96	16·29 ± 0·76
Control males	20	257·20 ± 5·48	12·15 ± 0·39
Females with diabetes insipidus	6	207·83 ± 12·45	37·73 ± 1·97
Control females	12	198·25 ± 5·08	31·10 ± 1·27

One difference is that the adrenal weights of male and female rats with either 'temporary' or permanent diabetes insipidus are greater than those of the glands from control animals; this is statistically significant ($P > 0·001$) (Table 1). Histological sections of these enlarged adrenals (Plate 1, nos. 1–6) show that the zona glomerulosa is narrow or absent. The zona fasciculata runs right up to the outer connective tissue capsule or, in other cases, a narrow zone of compact cells, probably



Plate 1

Photographs of part of the adrenal cortices of female rats in different experimental conditions (Bouin. H & E.)

1. Normal. The outer connective tissue capsule, the zona glomerulosa, the zona intermedia and the outer zona fasciculata can be seen (×150).

2. Diabetes insipidus. A narrow zona glomerulosa intervenes between the capsule and the hypertrophied zona fasciculata (×150).

3. Normal injected with 5 I.U. ACTH (long-acting Armour) per day for 3 days. A narrow zona glomerulosa intervenes between the capsule and the hypertrophied zona fasciculata (×150).

4. Part of (1). The normal zona glomerulosa (×440).

5. Part of (2). The narrow zona glomerulosa lying between the capsule and the zona fasciculata (×440).

6. Part of (3). The narrow zona glomerulosa obtained after ACTH injection (×440).

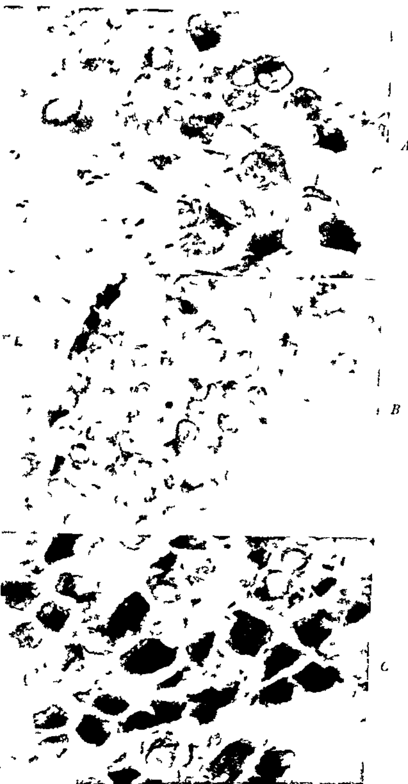


Plate 11

Effects of changes in salinity on the amount of neurosecretory material in the pars parvocellularis of the nucleus preopticus of *Ammodytes lanceolatus* (Bouin fuchsine paraldehyde acc. to Gabe). *A* = Control (in *normal sea water*). *B* = in concentrated sea water for one hour. *C* = one hour after return to normal sea water.

representing the zona glomerulosa and sometimes the zona intermedia intervenes (Plate I nos 2 and 5 Chester Jones & Wright 1954 Chester Jones 1956*b*) The same effect can be obtained by injecting ACTH into normal rats (Plate I nos 3 and 6) It seems that rising titres of ACTH in the intact animal are accompanied by enlargement of the zona fasciculata and by diminution of the zona glomerulosa This has been seen by other workers (Selye & Stone, 1950 Baker, 1952 Tonutti Bahner & Muschke 1954, Krohn 1955) On the basis of weight change and histology the adrenal cortex of the rat with diabetes insipidus may be regarded as hyperactive Baisset Douste Blazy Planel & Montastruc (1954) come to a similar conclusion for dogs with experimental diabetes insipidus

The consequences of adrenalectomy of the rat are well known and these must be due in large measure to the absence of corticosteroids In addition it has been suggested that the secretion of the posterior lobe of the pituitary and associated hypothalamic centres now uninfluenced by cortical hormones contributes to the enhanced renal reabsorption of water and to the increased sodium loss so characteristic of the adrenalectomized eutherian mammal (Gaunt 1951) On this hypothesis it was to be expected that differences might be found between on the one hand adrenalectomized control animals which have antidiuretic hormone but no adrenocortical hormones and, on the other adrenalectomized rats with permanent diabetes insipidus which have no or minimal antidiuretic hormone and no adrenocortical hormones Such proved to be the case (Table II) Control animals and rats

TABLE II

Changes in the sodium and potassium concentration of plasma and muscle following adrenalectomy of female rats with and without diabetes insipidus

(Means ± standard error)

Group	*n*	Body weight g	Plasma mEq /l Na	Plasma mEq /l K	Muscle mEq /kg wet wt Na	Muscle mEq /kg wet wt K	Muscle per cent water
Controls	12	190 63 ±4 94	148 36 ±4 22	4 96 ±0 22	23 39 ±0 31	100 26 ±0 99	75 43 ±0 33
Diabetes insipidus for 2 to 3 months	9	176 85 ±2 61	146 40 ±1 81	4 93 ±0 19	25 95 ±1 48	99 26 ±1 63	75 78 ±0 29
Controls adrenalectomized for 8 days	12	189 88 ±5 90	131 85 ±2 70	7 84 ±0 13	18 33 ±1 49	110 08 ±1 92	76 60 ±0 39
Diabetes insipidus adrenalectomized for 8 days	10	196 50 ±5 50	146 16 ±3 10	6 03 ±0 18	19 68 ±0 60	105 18 ±1 82	76 00 ±0 67

with diabetes insipidus had similar values for sodium and potassium content of plasma and muscle Control adrenalectomized animals showed a fall in sodium content and an increase in potassium content of plasma and muscle, generally found in the adrenalectomized eutherian Adrenalectomy of rats with diabetes insipidus *was not followed, however, by a fall in plasma sodium content the figure being of* the same order as that found in control animals, the amount of plasma and muscle potassium was increased above normal values though in neither was the increase as great as that occurring in the adrenalectomized controls The sodium content of the muscle of adrenalectomized rats with diabetes insipidus did not decline to the same extent as in adrenalectomized controls but the figure is clearly less than that for *normals The water content of muscle increased after adrenalectomy, a little more in* the adrenalectomized controls than in the adrenalectomized rats with diabetes insipidus, but this difference is not statistically significant This finding of normal sodium values for the blood of adrenalectomized rats with diabetes insipidus confirms those of Winter and his co workers (Winter, Gross & Ingram, 1938 Ingram & Winter, 1938 Winter Ingram Gross & Sattler 1941) for the cat adrenalectomized *after the establishment of diabetes insipidus It seems that the rat in the* absence of both adrenocortical and antidiuretic hormones is able to conserve sodium to some extent even in the face of a continued excretion of an appreciable amount of water the intake of the adrenalectomized rat with diabetes insipidus being about twice that of the normal animal per 24 hr (see below) It may be that the enzyme systems in the tubular epithelium concerned with the active transport of sodium can function without these two types of hormone which modify but do not initiate their activity Injection of posterior lobe hormones into adrenalectomized rats with diabetes insipidus brought about a decline in *plasma* sodium content but *in the doses used,* also produced terminal symptoms which are in any case associated with sodium loss Experiments to demonstrate whether oxytocin *or* vasopressin or both will lower the normal levels of plasma sodium in adrenalectomized rats with diabetes insipidus when still in good health are in progress

Further evidence for differences in capacity for sodium conservation between adrenalectomized controls and adrenalectomized rats with diabetes insipidus is given by 24 hr metabolic studies The rat with permanent diabetes insipidus eats about the same amount or a little more per 24 hr than the normal animal and drinks four to five times as much (Fig 1) After adrenalectomy of both kinds of animal the food intake declines sharply and then mounts slowly (Fig 2) and falls precipitously before *death The water intake of the diabetes insipidus animal drops to about half after* adrenalectomy (Fig 1) Adrenalectomy of control animals is followed by either unchanged water intake or a slightly increased one Both control and diabetes insipidus animals are in sodium and potassium balance Adrenalectomized controls have a negative sodium and a positive potassium balance In the adrenalectomized rat with diabetes insipidus there is some sodium loss up to 48 hr after operation this then becomes small, retention of potassium is not so marked as in the adrenalectomized controls Figure 2 in which the ratio of sodium to potassium excreted is plotted against time, illustrates this trend

The substitution of saline for tap water to drink keeps both the adrenalectomized and the adrenalectomized rat with diabetes insipidus in apparently good health Rats

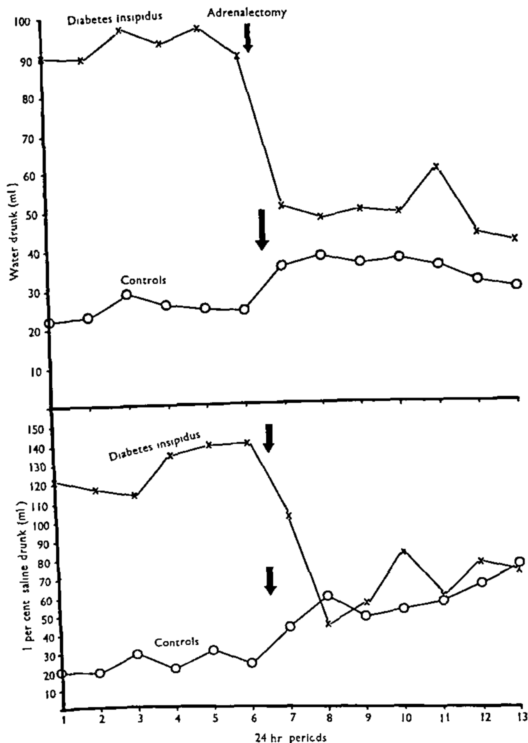


Figure 1

The amount of water and of saline drunk by normal rats and those with diabetes insipidus before and after adrenalectomy plotted against time

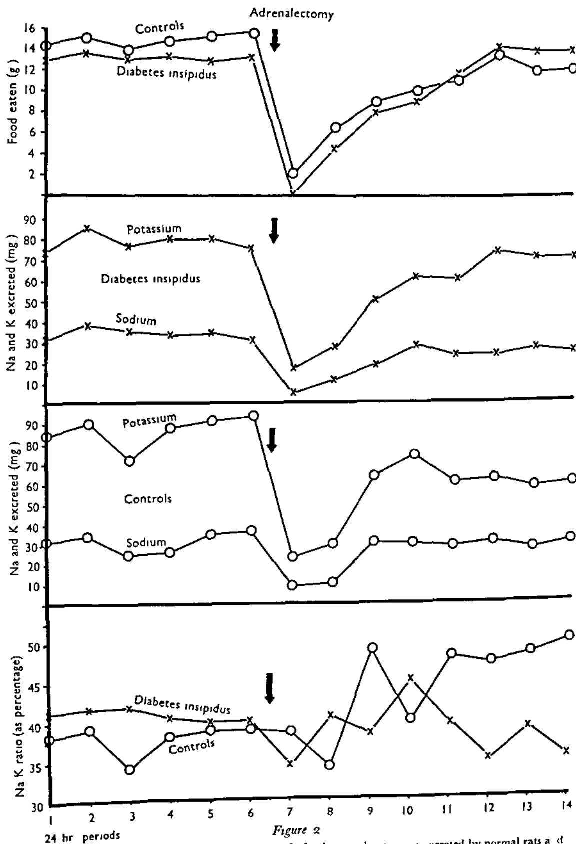


Figure 2

The amount of food eaten (commercial rat cake) and of sod um and potassium xcreted by normal rats a d those with diabetes insipidus before and after adrenalectomy plotted aga nst time the l ttom graph g ves the ratio of Na to K excreted under these cond tion

with diabetes insipidus drink large quantities of saline before operation and although the intake drops immediately after operation it then increases (Fig 1) and can reach high figures Most control animals drink about the same amount of saline as they do of tap-water but after adrenalectomy such animals match the adrenalectomized rats with diabetes insipidus in saline intake Constant ingestion of saline keeps the distribution of electrolytes in the body at or near normal levels presumably by osmotic means and in the tubule may facilitate potassium excretion It is probable that secretion of antidiuretic hormone in adrenalectomized controls has little relevance in the reactions to saline ingestion In both types of animal the paramount factor is the absence of adrenocortical hormones the rise in glomerular filtration rate the large amount of sodium transported by the renal tubule and the tendency to dehydration

The reactions of an animal to daily intake of food and water offered freely and those to an administered load differ widely Loading rats by stomach tube with different solutions is a method whereby some aspects of hormonal competence can be assessed against a background of renal dynamics not themselves easy of resolution I used the method of giving either water or hypertonic saline or hypertonic potassium chloride to various categories of rats Details including doses are given by Boss, Birnie & Gaunt (1950) and Chester Jones & Spalding (1954) In all three types there was a priming dose of water followed one hour later by either water or saline or potassium chloride These loads were given to the following categories of animals (i) controls that is animals with antidiuretic hormone and adrenocortical hormones (ii) rats with permanent diabetes insipidus with no antidiuretic hormone (or minimal quantities) but with adrenocortical hormones (iii) adrenalectomized controls that is animals with antidiuretic hormone but no adrenocortical hormones (iv) adrenalectomized rats with diabetes insipidus with no antidiuretic hormone or adrenocortical hormones The results are given in Table III It should be noted that adrenalectomized animals of both kinds have a slower rate of absorption from the gut (so that in the first hour normal animals have absorbed 50 per cent of the water load while adrenalectomized animals have absorbed 22 8 per cent) a lower blood pressure and a slower glomerular filtration rate than unadrenalectomized animals (Table III) This means that the actual rate of presentation of filtrate to the nephron is less than in normal animals The following points may be made from examination of Table III

I *The consequences of loading with water*

(1) Normal rats and rats with diabetes insipidus excrete water at similar rates and have much the same G F R

(2) Both adrenalectomized controls and adrenalectomized rats with diabetes insipidus have equal difficulty in excreting water this despite the absence of detectable antidiuretic substances in the blood of the latter when the method of Birnie Eversole Boss Osborn & Gaunt (1950) was used although the blood of adrenalectomized animals does show antidiuretic activity after this method (Birnie *et al* 1950 Ames & van Dyke 1952 Ginsburg 1954)

(3) Adrenalectomized animals excrete little urine but this is high in sodium content Under these conditions any capacity possessed by adrenalectomized rats with diabetes insipidus to conserve sodium beyond that of adrenalectomized controls is not

T

TABLE III

The output of urine sodium and potassium and the glomerular filtration rate (G F R) of normal male and female rats rats with permanent diabetes insipidus rats adrenalectomized for 4 days and rats with permanent diabetes insipidus adrenalectomized for 4 days after loads (calculated at 3 ml /100 cm surface area) by stomach tube of (i) distilled water or (ii) hypertonic saline (5 mg /ml) or (iii) hypertonic potassium chloride (5 mg /ml) The blood pressure (taken in urethane anaesthesia) is also given for the four categories of animals

(Means ± standard error)

	n	Urine ml /100 g min	Na micro eq /100 g / min	K micro eq /100 g / min	G F R ml /100 g / min	Blood pressure mm Hg
Water loading						
Controls	29	0 036 ± 0014	0 053 ± 0045	0 145 ± 019	0 600 ± 059	108 9 ±2 7
Diabetes insipidus	10	0 034 ± 0025	0 049 ± 0037	0 127 ± 009	0 581 ± 114	96 0 ±2 7
Adrenalectomized	30	0 0037 ± 0005	0 320 ± 053	0 101 ± 012	0 103 ± 016	5° 5 ±1 5
Diabetes insipidus and adrenalec tomized	12	0 0026 ± 0004	0 314 ± 077	0 097 ± 009	0 112 ± 026	55 9 ±1 3
Hypertonic saline loading						
Controls	19	0 023 ± 0016	2 81 ± 27	0 362 ± 029	1 289 ± 181	
Diabetes insipidus	12	0 046 ± 0029	5 94 ± 17	0 399 ± 0069	1 407 ± 16	
Adrenalectomized	12	0 016 ± 006	1 87 ± 137	0 134 ± 012	0 143 ± 024	
Diabetes insipidus and adrenalec tomized	9	0 014 ± 004	1 36 ± 26	0 126 ± 028	0 112 ± 023	
Hypertonic potassium chloride loading						
Controls	30	0 031 ± 002	1 17 ± 09	2 96 ± 03	2 75 ± 49	
Diabetes insipidus	9	0 042 ± 004	1 32 ± 14	2 70 ± 12	3 14 ± 46	

revealed Presumably (i) water ingestion does not tend to stimulate secretion of antidiuretic hormone in adrenalectomized controls (ii) the kidneys react differently when the animal is coping with administered loads and when it responds to eating and drinking *ad libitum*

II *The consequences of loading with hypertonic saline*

(1) The administration of hypertonic saline clearly differentiates between control animals and those with diabetes insipidus (see Hare Hare & Philips 1943 Hickey & Hare 1944) The urine output of normal animals is reduced compared with that obtained after the administration of an equivalent volume of water The basis of this is the release of antidiuretic hormone under the influence of the increased effective osmotic pressure of the blood (Verney 1947) The rats with diabetes insipidus show a diuresis not only of water but also of sodium The output of potassium is about the same in normal and in diabetes insipidus rats it is increased beyond that obtained after water loads because of the increased G F R brought about by hyper tonic saline administration

(2) Adrenalectomized rats given hypertonic saline do not show this significant increase of G F R but the output of urine is increased above that given after water loads Bristol & Drill (1952) have shown that the response of adrenalectomized rats to saline loads depends on the strength of solution and the time after operation it is administered and one factor involved must be the capacity of the saline to alter glomerular filtration rate Though both types of adrenalectomized animals have the same order of G F R and excrete similar amounts of potassium (Table III) the output of sodium in adrenalectomized animals with diabetes insipidus is significantly smaller than that in adrenalectomized controls It may be that under these conditions the absence of antidiuretic hormone allows of greater sodium reabsorption while anti diuretic hormone uninfluenced by corticosteroids actually enhances sodium ex cretion Nevertheless the adrenalectomized rat with diabetes insipidus without antidiuretic hormone or adrenocortical hormones cannot respond to hypertonic saline by a water and sodium diuresis in the same way as the rat with diabetes insipidus and intact adrenals

III *The consequences of hypertonic potassium chloride loading*

(1) Hypertonic potassium chloride produces a good urine flow in both normal and diabetes insipidus rats the former do not show such a prompt diuresis in hour 1 as do the latter The glomerular filtration rates in both cases are considerably increased above those figures given after water and saline loading The excretion of potassium is at a high rate in both types of animal Of particular interest is the vast amount of sodium excreted though sodium was not administered The amount is about 20 times that given after water loading though the G F R with potassium chloride loading was increased only 4½ times Presumably potassium and sodium compete for hydrogen ion in a cation exchange mechanism (Berliner 1952) With the flooding of the renal tubule by potassium and its presumably copious re secretion there is reduced hydrogen ion secretion and this is mirrored in the alkalinity of the urine

after potassium ingestion With less hydrogen ion available the normal reabsorption of sodium is lessened and more appears in the urine This may operate without positive hormonal action On the other hand rats with relative adrenal insufficiency after adrenal enucleation and regeneration (Chester Jones & Spalding, 1954, Chester Jones 1956a) excrete water (0 027 ±0 0012 ml /100 g /min) and potassium (2 65 ±0 087 micro eq /100 g /min) at about normal rates but sodium at a diminished rate (0 543 ±0 073 micro eq /100 g /min) (compare values in Table III) This may illustrate the need of adequate corticosteroid secretion both for reabsorption and for excretion of sodium, a paradox of the actions of adrenocortical hormones

When we talk about adrenocortical hormones what hormones do we mean? The rat secretes corticosterone and aldosterone other animals the latter and varying proportions of Kendall s compounds *B* and *F* among other steroids (Hechter & Pincus, 1954) I have recently started some experiments in which rather than give a load by stomach tube I have infused rats intravenously at a constant rate after external jugular vein cannulation (Ginsburg & Heller 1953) My experiments are in the preliminary stages and so far I have only infused rats with hypertonic saline, chosen because it gives such an obvious difference between normal and diabetes insipidus rats Thus during hour 1 of the infusion of 1 2 per cent saline at 0 1 ml /min rats with diabetes insipidus do not put out a great deal more urine than normal animals (Fig 3) In hour 2 and subsequently, however, while normal rats reach a steady state the output of urine in diabetes insipidus rats is rather more than the volume of saline infused and indeed the diabetes insipidus rat loses about 2 per cent of its body weight over a 4 or 5 hour infusion period At the same time the output of sodium and potassium in normal and diabetes insipidus rats is about the same rats with diabetes insipidus not showing a sodium diuresis as they did when hypertonic saline was given by stomach tube Aldosterone in a 3 microgramme dose given slowly intravenously at the end of hour 1 (Fig 3) depressed the urine output of animals with diabetes insipidus This increased renal reabsorption of water seems to be correlated with the increased reabsorption of sodium which is also manifested Aldosterone then, does not seem to have any direct effect on the rate of water excretion in the doses used (Simpson & Tait 1955) Similarly intravenous injection of aldosterone at hour 3 into adrenalectomized rats continuously infused with hypertonic saline does not alter the amount of urine excreted compared with normal rats under these conditions On these grounds, it would be supposed that the hyperactive adrenal of the rat with diabetes insipidus is not secreting increased amounts of aldosterone but of corticosterone It might be argued that corticosterone in rats and 17 hydroxycorticosterone in some other animals has a positive diuretic effect contributing at the tubular level to the excretion of water by as one conceivable mechanism altering the permeability to water of the epithelium (as discussed by Dr Wirz in this symposium) There is evidence that cortisol and cortone will cause a diuresis in adrenalectomized animals and in the Addisonian and that this effect is beyond that due to increased glomerular filtration rate But cortisol can itself cause sodium retention and under certain conditions such an action may be accompanied by increased water reabsorption Paradoxes arise because corticosteroids can only act within the limits of the renal dynamics at the time their actions are in part determined by the composition of the filtrate in the renal tubule

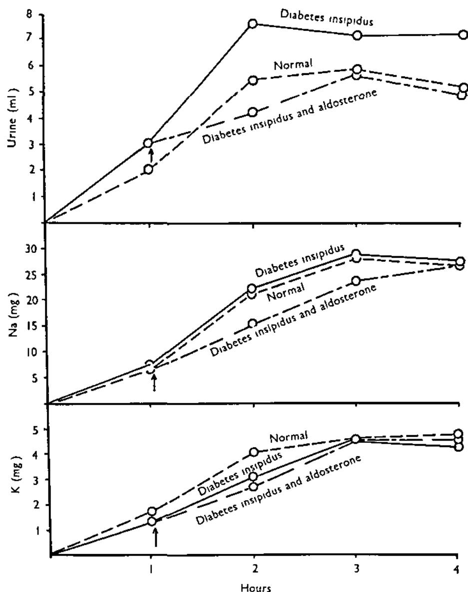


Figure 3

The urine, sodium and potassium output of normal rats and rats with diabetes insipidus infused intravenously with hypertonic saline (1·27 per cent.) at the rate of 0·1 ml. per min. for 4 hours. Aldosterone (3 µg.) was infused slowly at the end of hour 1 into rats with diabetes insipidus. The marked water diuresis of rats with diabetes insipidus following hypertonic saline was suppressed by aldosterone consequent on the accompanying sodium retention.

Other difficulties arise from the complex nature of the mechanisms apparently controlling ACTH release. Workers who use changes in urine output as indicative of antidiuretic hormone action alone are ignoring the factors *introduced* by corticosteroid secretion which may indeed be enhanced by the very test procedures used

Thus, the manipulation necessary to give fluids by stomach tube causes a depletion of adrenal ascorbic acid content (Nowell, unpublished) The degree of this depletion is about the same with insertion of the stomach tube without giving a solution as with loading with hypertonic saline or with Ringer's solution isotonic to the body fluids This means that results obtained by loading by stomach tube are affected by the handling of the animal and this must alter the results—in an unknown way Especially so as we do not know what depletion of adrenal ascorbic acid content means in terms of the hormones secreted by the adrenal cortex With the apparent multiplicity of types of corticotrophin and of the kinds of corticosteroids not all being equally dependent on release by ACTH, this problem may be regarded as a little confused But as aldosterone is at least in part if not wholly independent of ACTH secretion

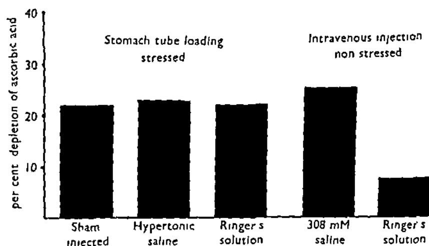


Figure 4

The depletion of adrenal ascorbic acid (estimated by Roe & Kuether's (1943) method) following use of the stomach tube and of intravenous injection While the manipulation itself associated with the insertion of the stomach tube caused marked depletion it was possible to give 6 ml Ringer's solution slowly intravenously with only a small (7 per cent) fall in adrenal ascorbic acid On the other hand 6 ml of hypertonic (twice isotonic) saline effected a marked depletion The infusion of hypertonic saline presumably called forth antidiuretic hormone secretion and this may be a factor accounting for the difference in response in terms of adrenal ascorbic acid change between this solution and the isotonic one (From Nowell unpublished)

it would be reasonable to suppose that in the rat fall in adrenal ascorbic acid content is associated with increased corticosterone production We have found, however, that it is possible to administer solutions to rats without causing stress by the very process of injection (Nowell & Chester Jones unpublished) Rats with the external jugular vein cannulated can be given solutions through the cannula without awareness of the procedure by the rat in terms of variation in adrenal ascorbic acid content Using this experimental approach it was found that hypertonic saline (twice isotonic) delivered intravenously caused a marked fall in adrenal ascorbic acid content while the same volume of isotonic Ringer solution caused only a small drop (Fig 4) This may mean that (1)—the addition of hypertonic saline to the blood caused the secretion of antidiuretic hormone which in turn is an effector in the release of ACTH This then would be the *obligatory release of ACTH* That is conditions which release

antidiuretic hormone (or a substance accompanying it?) must of necessity, irrespective of the renal needs of the animal release ACTH to activate the adrenal or that (ii)—hypertonic saline injection results in the need for the animal to excrete sodium The renal mechanisms involved require corticosteroids and the corticotrophin adrenocortical hormone mechanism is activated This would imply increased secretion of corticosterone rather than any change in aldosterone secretion which may be diminished

It is an interesting development of recent researches that the hypothalamic factor which is responsible for secretion of ACTH has been equated with the antidiuretic hormone or to a similar factor contained within commercial vasopressin (Pitressin) (see Martini & Morpurgo, 1955 Gaunt *et al* above) If we consider the reaction of the rat with diabetes insipidus to stress we are examining an animal with no (or little) antidiuretic hormone While control animals placed in the cold at 30 F for 1 hour show a marked fall in adrenal ascorbic acid content rats with diabetes insipidus show only a small drop of adrenal ascorbic acid content under these conditions (Fig 5) Furthermore injection of vasopressin in a dose of 0 3 U half an hour before exposure to cold allows the adrenal ascorbic acid content to fall markedly approaching though not equalling control figures (Fig 5) Sham injection does not have this effect nor does

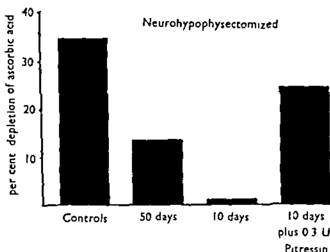


Figure 5

The change in adrenal ascorbic acid content of rats when exposed to 30 F for 1 hour Normal animals showed a 34 2 per cent depletion of adrenal ascorbic acid Rats with diabetes insipidus for 10 days showed little depletion and those with diabetes insipidus for 50 days rather more but still only 14 per cent depletion The injection of 300 mU Pitressin half an hour before exposure to cold of rats neurohypophysectomized for 10 days was followed by a 24 per cent depletion of adrenal ascorbic acid content (From Nowell unpublished)

it occur in hypophysectomized animals it seems therefore that something in Pitressin elicits ACTH release The fact that the diabetes insipidus rat does not react to cold stress as does the normal animal may be due to (i) the lack of antidiuretic hormone (or an associated substance) so that the effector for the release of ACTH is absent or (ii) since the diabetes insipidus rat has a hyperactive adrenal in any case the animal

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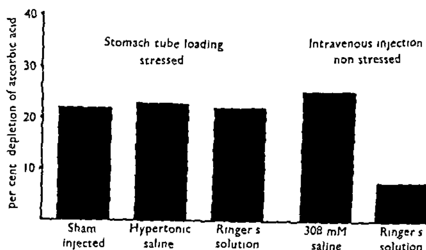


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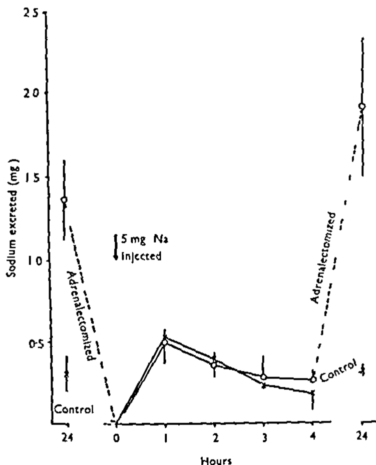


Figure 6

The loss of sodium in 24 hr from normal and from adrenalectomized winter frogs (9 of each) when kept in distilled water. At zero, 5 mg Na (as frog Ringer's solution) was injected into the dorsal lymph-sac and the sodium excretion over the first 4 hr period and then over 24 hr is given. During the latter the excretion of sodium from the adrenalectomized frogs is the more marked (points shown with standard deviations). (From Fowler, 19[illegible])

it loses sodium at a faster rate than control animals when placed in distilled water for 24 hr (Fig 6) When 5 mg Na (as frog Ringer's solution) was injected into the dorsal lymph-sac there was little difference over the first 4 hr in the rate of sodium excretion between adrenalectomized and control animals Over 24 hr, however, the sodium loss was much greater from the adrenalectomized animals and indeed, the secretion rate of the control did not vary greatly from that occurring before the injection of the sodium load (Fig 6) Thus sodium loss, following adrenal removal, is characteristic both of the amphibian and the mammals Saline replacement therapy too is successful in both cases For it is possible to maintain adrenalectomized summer* frogs which normally die 24 to 4 hr after operation, in an active state by immersion in saline Frog Ringer's solution is moderately and 0 73 per cent saline (Aldred

can cope with cold stress without further demand for corticosteroids and hence for the release of more ACTH Experiments are in progress aimed to elucidate these points

OTHER VERTEBRATES

When we turn to the consideration of other classes of vertebrates there are at least five groups of questions to which we must seek the answers

(i) What adrenocortical steroids are produced in the different classes of vertebrates?

(ii) What tissues do the adrenocortical hormones affect do they all influence the kidney epithelium, whether it be meso or metanephros? Are they chiefly preoccupied with water and salt electrolyte metabolism and does their scope include the gill epithelium of the fish and the skin of the amphibian and the selective permeability of the membrane of tissue cells?

(iii) Do the adrenocortical hormones interrelate with the actions of the neuro hypophysial *principles which seem to be concerned at least in part with water* metabolism in those vertebrates in which a physiological role has been assigned to them?

(iv) Does an adenohypophysial corticotrophin regulate the secretions of the adrenal cortex in all vertebrates *if so does the ACTH control all or only part of the adrenal* cortex and its secretions?

(v) What are the mechanisms whereby ACTH is released and do the posterior lobe of the pituitary and the hypothalamus play a part in the control of anterior lobe *functions*?

The answers to these questions are not known Nor can all the possibilities be discussed here Some account of them has been given elsewhere (Chester Jones, 1956*a* *b*) I should like however to discuss some results Fowler has obtained using the frog, *Rana temporaria* (Fowler & Chester Jones, 1955 Fowler 1956) Because our knowedge is so limited we are predisposed to think of amphibian physiology in terms of the mammalian As we survey both classes the similarities of water and salt electrolyte metabolism are striking Both the neurohypophysial and the adrenocortical hormones *seem to be capable of changing the extent of epithelial absorption of water* and of sodium whether it be in the skin or in the kidney and whether the effects be primary or secondary ones The problem is on the one hand one of the varying tissue permeability to water occasioned perhaps by changing pore size and on the other the varying rate of sodium transport dependent on some enzyme system While the action of neurohypophysial hormones in amphibians has been investigated many times (see Sawyer, 1956 and in this Symposium), the role of the adrenal cortex in amphibian water and salt electrolyte metabolism has been little followed Though the adrenalectomized winter* frog (Chester Jones 1956*a*) has no outward signs of ill health

* *Rana temporaria* is of the winter type from September to December inclusive eating avidly at first and then sparsely Frogs of the summer type cover the pre spawning and spawning periods of January to March inclusive the post spawning period of April and the true summer frog of May to August inclusive when the frogs eat lay down fat and their gonads regenerate Broadly speaking the winter frog is metabolically inactive *the summer frog* metabolically active

TABLE V

The effects of adrenalectomy of adenohypophysectomy and of hypophysectomy on the body weight and on the water sodium and potassium content of muscle of frogs

(Means ± standard error) Fowler 1956

Frogs	n	Body weight Exptl period		Muscle				
		Start	End	Percentage water	mEq/kg Wet weight K	mEq/kg Wet weight Na	mEq/kg Dry weight K	mEq/kg Dry weight Na
Winter Controls	12		23 70 ±2 02	77 95 ± 41	80 54 ±1 55	25 00 ± 54	366 11 ±2 80	113 95 ±4 10
Adrenalectomized 14 days	8	25 51 ±1 79	24 65 ±1 52	79 49 ±2 04	73 91 ±2 18	14 50 ±2 00	363 11 ±10 60	71 08 ±3 44
Adenohypophysectomized 14 days	12	27 92 ±2 37	27 63 ± 35	80 54 ±1 55	76 41 ± 82	17 20 ± 95	394 44 ±9 69	88 66 ±7 41
Summer Controls	9		24 90 ±1 79	80 65 ± 31	83 66 ±1 75	25 81 ±1 36	435 55 ±5 37	133 2 ±2 10
Adrenalectomized 24–48 hr	8	27 87 ±1 87	30 87 ±2 15	86 50 ±1 49	62 50 ±2 00	9 30 ± 34	462 98 ±8 41	68 21 ±3 46
Sham adrenalectomized showing oedema	5	26 15 ±1 38	8 45 ±2 00	86 96 ±1 11	54 60 ±1 33	7 96 ±1 70	406 12 ±7 4	64 32 ±5 10
Adenohypophysectomized 24–48 hr	9	24 59 ±1 45	29 5 ±1 96	86 50 ±1 10	59 90 ±2 00	12 34 ±3 1	446 77 ±12 54	91 36 ±7 03
Hypophysectomized 24–48 hr	10	2 02 ±2 27	25 47 ±1 37	88 10 ± 61	48 00 ±2 60	16 20 ±1 40	414 33 ±14 33	136 33 ±4 00

content does not change with the loss of sodium in adrenalectomized and adenohypophysectomized animals It was interesting to find that when both lobes of the pituitary were removed (hypophysectomy) loss of sodium (per kg dry weight of muscle) was not apparent though other symptoms of adrenal insufficiency occurred Though more work is required on this point the situation is reminiscent of the adrenalectomized rat with diabetes insipidus which showed some capacity for sodium conservation beyond that of the adrenalectomized control We may suppose that the loss of sodium in the frog is manifested because the normal tubular reabsorption of sodium which must accompany the production of a hypotonic urine cannot go on in the absence of adrenocortical hormones The posterior lobe hormones probably influence not only glomerular filtration rate but also tubular reabsorption of water It is conceivable that this latter similarity to the position in mammals may also be joined by the action of neurohypophysial hormones on sodium excretion in both

1940) is very effective in this respect The latter solution allows the sodium and potassium muscle content of adrenalectomized animals to be within the normal range (Table iv)

TABLE IV

The maintenance of adrenalectomized summer frogs with saline solutions

(Means ± standard error) Fowler 1956

Summer frogs	*n*	Body weight: Exptl period: Start	Body weight: Exptl period: End	Muscle: Percentage water	Muscle: mEq/kg Wet weight: K	Muscle: mEq/kg Wet weight: Na	Muscle: mEq/kg Dry weight: K	Muscle: mEq/kg Dry weight: Na
Controls (kept in tap water)	9		24 90 ±1 79	78 76 ± 58	81 73 ±1 41	23 47 ±1 41	425 33 ±4 33	121 88 ±2 38
Controls (kept in frog Ringer's soln)	9	21 11 ±0 36	19 24 ± 92	83 25 ± 37	86 67 ±2 48	24 29 ±1 68	526 00 ±10 10	148 00 ±3 87
Adrenalectomized 24-48 hr (tap water)	8	27 87 ±1 87	30 87 ±2 15	86 5 ±1 49	62 50 ±2 00	9 30 ± 34	462 98 ±8 41	68 1 ±3 46
Adrenalectomized 5 days (Ringer's soln)	8	25 87 ±1 48	29 40 ±1 70	80 53 ± 79	75 71 ±1 45	12 17 ± 68	441 44 ±4 33	96 88 ±3 03
Adrenalectomized 5 days (*0 73 per cent saline*)	9	20 60 ±1 48	19 99 ±1 64	78 08 ± 21	92 21 ±1 06	26 34 ± 64	425 00 ±2 02	101 00 ± 88

A further point is revealed by these experiments namely that there is an increase in body weight consequent on adrenalectomy due apparently to increased uptake of water through the skin There is no weight increase when isotonic saline bathes the skin the passive movement of water not occurring in the absence of osmotic difference between the internal and external media Adrenalectomy, then can be followed *in the active summer frog at least by water uptake characteristic of the Brunn* effect obtained by the injection of neurohypophysial extracts into intact frogs (Brunn 1921, Heller 1956 Sawyer 1956) Sham operations not only of adrenalectomy but also of hypophysectomy sometimes also produce oedema and this may well be due to adrenocortical exhaustion rather than the result of cutting the skin which in any case does not apply to pituitary removal (Table v) It may be supposed that the adrenocortical steroids alter the permeability of frog skin to water and conjoin in this effect with the posterior lobe secretions

The loss of sodium from the muscle of both adrenalectomized winter and summer frogs also occurs when the anterior lobe of the pituitary (adenohypophysectomy) is removed (Table v) The extent of the changes in sodium and potassium are misleading when expressed *in terms of wet weight of muscle because of the increase in* muscle water content In terms of muscle dry weight it can be seen that muscle potassium

of deoxycortone acetate by change in the water sodium and potassium content of muscle (for the duration and in the doses given) though the histological picture of the adrenal cortex is one of degeneration (Table VI) Clearly further data on the nature of adrenal secretion in the lower vertebrates are required

Surveying what we know about adrenocortical function in vertebrates—and this is very little—the hypothesis may be advanced that these hormones act by altering the permeability of some epithelia and cell membranes generally to water and by changing the rate of the active transport of sodium in certain tissues Neurohypophysial secretions may join with adrenocortical hormones certainly in the first and doubtfully in the last possibility It may be supposed then that various parts of the vertebrate nephron are rendered less permeable to water in the presence of adrenocortical hormones and more permeable in their absence The actual passive movement of water may be conceived as depending on the epithelial permeability plus the filtrate concentrations in the tubule at the same time, these latter in turn depending among other things, on the rate of the active transport of sodium The gill epithelium of the bony fish and the skin of the frog may both be influenced by these hormones the passage of water through them being a passive process but the rate being conditioned by varying permeability under hormonal control But though it can be shown that the amphibian responds to posterior lobe principles the fish has so far resolutely refused to display water and salt electrolyte changes with injection of these secretions Indeed the only demonstration of the activity of the posterior lobe in fish is that of Wilhelmi Pickford & Sawyer (1955) who showed that fish and mammalian neurohypophysial preparations induced the spawning reflex response in *Fundulus* the killifish Should this be confirmed as a general mechanism in all bony fish and so far we have failed to confirm it in trout it might change our concepts of seeking the evolutionary change of posterior lobe function from that of water metabolic control to that of regulation of some aspects of reproduction

Here the action of the neurosecretions would be central rather than on peripheral tissue It would follow that the hypothalamic control of the anterior lobe of the pituitary by some substance of the nature of the neurohypophysial neurosecretions is the major mechanism appearing early in the evolution of the vertebrates The water balance effect and the antidiuretic hormone could be considered on this account later evolutionary manifestations of the main central apparatus Since, however one prime need of the vertebrate was and is osmoregulation it may be supposed that the adrenocortical secretions are active in this process throughout the vertebrates Adrenocortical secretions require corticotrophin control and this anterior lobe hormone may in turn be regulated by the hypothalamic neurosecretion So whether we consider the neurohypophysis to have activity primarily in the field of reproduction and secondarily in water and salt electrolyte metabolism or the reverse of this we arrive back at the hypothalamus and its possible role in the regulation of various hormonal activities and it is here that further research will be rewarding not only in mammals but in the lower vertebrates

ACKNOWLEDGEMENTS

The investigation of rats with diabetes insipidus was helped by a grant from the Medical Research Council investigation of frogs and snakes was aided by a grant

classes though even in mammals their influence on salt electrolyte metabolism has not been shown to be of normal physiological occurrence Change in sodium outflux through the skin is an additional complicating factor in amphibia

Though we discuss frog adrenocortical hormones, what these are is not known There are a few clues to their general identity Mammalian ACTH injected into hypophysectomized frogs will maintain the sodium content of muscle at about normal values (Table VI) and we may suppose that steroids akin to those of the eutherian

TABLE VI

The effects of the injection of mammalian ACTH and of deoxycortone acetate on the water sodium and potassium content of the muscle of normal and adenohypophysectomized summer frogs (Fowler 1956) and on that of the snake (Natrix natrix) (Wright unpublished)

	n	Body weight		Muscle				
		Exptl period		Per centage water	mEq /kg Wet weight		mEq /kg Dry weight	
		Start	End		K	Na	K	Na
Summer frogs								
Controls sham injected	9	23 37 ±1 02	22 29 ±1 33	78 76 ± 63	77 79 ± 41	25 32 ±1 24	366 33 ±3 24	118 88 ±9 05
ACTH (1 I U /day for 10 days)	7	20 30 ± 98	19 53 ±1 04	79 87 ± 77	67 90 ±1 04	30 40 ±1 08	339 00 ±4 71	151 00 ±3 10
DCA (2 0 mg /day for 10 days)	12	20 14 ± 77	19 20 ± 68	79 81 ± 46	76 37 ±2 23	24 76 ±1 00	378 55 ±4 86	122 45 ±1 56
Adenohypophysectomized 24-48 hr	9	24 59 ±1 45	29 25 ±1 96	86 50 ±1 10	59 90 ±2 00	12 34 ±3 10	446 77 ±12 54	91 36 ±7 03
Adenohypophysectomized and ACTH (2 I U / day for 5 days)	5	17 10 ±1 14	15 10 ±1 45	82 00 ± 55	74 60 ±1 37	28 95 ±1 39	414 66 ±4 18	160 44 ±2 60
Snakes								
Controls sham injected	9	71 70 ±4 40	70 60 ±4 40	79 50 ± 44	86 30 ±3 00	51 40 ±4 70	420 00 ±14 50	230 00 ±21 00
DCA (0 5 mg /day for 11 days)	8	64 52 ±4 51	62 01 ±3 20	78 24 ± 64	83 92 ±1 41	48 81 ±1 22	383 43 ±6 40	224 12 ±5 51

adrenal were produced to effect this Moreover we have made a tentative identification of Kendall s compound *B* in adrenal blood of birds and snakes (Phillips unpublished) The amphibian adrenal may secrete this compound too but a steroid similar to aldosterone would also be expected We have not had as yet adequate samples of adrenal blood to identify aldosterone in the lower vertebrates It is interesting to find however, that both frogs and snakes do not respond to the injection

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Discussion

Chairman H B van Dyke

L Arvy I would like to report some work done at the Sorbonne in collaboration with Dr Gabe on two species of marine teleost before and after the fishes had been placed in saline solutions of various concentrations

If one take *Callionymus lyra* or *Ammodytes lanceolatus* from normal sea water and examines paraffin sections of the hypophysis after staining with Gomori s chrome haematoxylin phloxin or by the aldehyde fuchsin technique of Gabe one sees significant quantities of neurosecretory substance In the same species after a very short time (as little as thirty minutes) in concentrated sea water very pronounced depletion of the neurosecretory substance can be observed if the fishes are returned to normal sea water for one hour the hypophysis recovers its normal content of neurosecretory material If *Callionymus lyra* or *Ammodytes lanceolatus* are put in dilute sea water their hypophyses contain more neurosecretory product than usual

Plate II shows the pars parvo cellularis of the nucleus preopticus of *Ammodytes lanceolatus* When the fishes are kept in normal sea water the neurosecretory cells are full of secretion and the axons are also rich in this material which forms classical Herring bodies In fishes which had been subjected to concentrated sea water for

from the Nuffield Foundation for work in the field of Comparative Endocrinology Dr M A Fowler, now at the Department of Biology, Guy s Hospital Medical School, did the work on frogs while holding a Medical Research Council studentship, and Dr A Wright now at the Department of Physiology and Histology, Liverpool University that on snakes (only briefly mentioned here) while in receipt of, in the first place, a Nuffield and later, an Agricultural Research Council Grant Dr N W Nowell was working in this laboratory while on leave of absence from the Department of Zoology University of Hull I wish to thank Mrs M H Lawson for technical assistance

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retention of salt in his adrenalectomized rats after an intravenous salt load or at least he found that they excreted much the same amounts as untreated adrenalectomized rats

Chester Jones Intravenous infusion of salt into adrenalectomized rats was done four days after adrenalectomy when the adrenal insufficiency would be fairly well established yet the animals strong enough to withstand manipulation The slide I showed was to demonstrate the lack of effect of aldosterone on water excretion in adrenalectomized rats intravenously infused with hypertonic saline sodium excretion was not shown on the graph Aldosterone produced marked sodium retention when given to rats with diabetes insipidus intravenously infused with hypertonic saline The sodium retention so produced is accompanied by an increased reabsorption of water and this is the explanation I offered for the lessening by aldosterone injection of the osmotic diuresis of diabetes insipidus rats receiving hypertonic saline

Gaunt If you had used a plain water load and then given aldosterone you would have got enhancement of water diuresis The doses of aldosterone required however are about the same as those of hydrocortisone

Lloyd May I ask Dr Stutinsky what happens to the supraoptic and paraventricular nuclei in diabetes insipidus animals which are given salt

Stutinsky There is a depletion of neurosecretory material

Lloyd Were these animals maintained on salt for a long time?

Stutinsky Nine days

Lloyd Is there degeneration of the nuclei?

Stutinsky I cannot say

one hour, the neurosecretory cells lost most of their neurosecretory product In fishes which had then been returned to normal sea water for one hour, the neurosecretory cells were again full of secretory material and had big lobulated, convoluted and multinucleolated nuclei in short, they showed many cytological signs of great secretory activity Similar changes in the amounts of neurosecretory material were found in the pars magno cellularis and in the neurohypophysis

If the hypothalamo hypophysial tract of fishes subjected to concentrated sea water is stained by Mann s method, marked hyperaemia can be seen, blood vessels are enlarged and filled with red cells It seems that the hyperaemia is correlated with the rapid migration of neurosecretory material from the nucleus preopticus to the neuro hypophysis and from there to the blood

In conclusion, concentrated sea water provokes a rapid decrease in concentration of neurosecretory material in the neurosecretory cells of the nucleus preopticus and in the hypophysis the return to normal sea water is followed by restoration of the normal appearance Dilute sea water provokes an increase in the concentration of neurosecretory material in the nucleus preopticus and the hypophysis In each case the blood supply to the hypothalamo hypophysial tract is greatly modified

Stutinsky It is a well known fact that in rats experimental diabetes insipidus due to the removal of the posterior pituitary is more severe if the animals drink hypertonic salt solutions The question arises—what happens in the adrenals of these rats? We have found that there are no marked changes in the zona glomerulosa but the modifications seen in the fasciculata are very important Many cells contain colloid like droplets of varying sizes some as large as and even larger than a nucleus The whole fasciculata shows many of these cells and colloid droplets which can also be seen in cells of the reticularis The droplets can be stained with Azan or Masson's stain They do not stain with Baker s acid haematin or Sudan Black but they stain remarkably well with the periodic acid Schiff technique I would suggest tentatively, that in normal rats drinking hypertonic saline the secretions of the normal neural lobe protect the zona fasciculata against these morphological modifications

Gross Dr Loustalot in our laboratories has seen this material in rats adrenals quite often, and not only in animals treated with high doses of methylandrostenediol When Selye produced hypertension with methylandrostenediol, he found these droplets regularly in the adrenals and claimed that they were specific for an increased production of mineralocorticoids but you may also find the same material in normal animals especially in senile or poisoned ones Rats of more than two years of age have been investigated by Dr Loustalot and these droplets have been seen quite regularly, but not on such a scale as in animals treated with high doses of DCA and salt We think that these droplets are a sign of non specific degenerative changes in the adrenals

Stutinsky I agree that this is not a specific effect

Gross I would like to ask Dr Chester Jones whether in his experiments with adrenalectomized rats, the rats were really insufficient or did he do the experiments only one or two days after adrenalectomy? If I understood him correctly, he also found no